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ON THE CYTOLOGY OF THE VEGETATIVE AND REPRODUCTIVE ORGANS OF THE SAPROLEGNIEÆ.

By MARCUS HARTOG, M.A., D.Sc., Professor of Natural History, Queen's College, Cork.

(Plates XXVIII. and XXIX.)

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XVII.

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(Plates XXVIII. and XXIX.)

[communicated by W. J. SOLLAS, LL.D., F.R.S.]

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Section 1.—Literature of the Hyphæ and Zoosporange.

The literature of this subject is not very extensive. While spore-formation and the protoplasmic currents in this group were the frequent object of study by the older writers, Schmitz, in his pioneering research, "Die Zellkerne der Thallophyten," was the first to demonstrate the nuclei of the Phycomycetes in 1879. He describes them* as roundish bodies embedded in the parietal protoplasm, multiplying by binary fission, and closer together in the younger parts than in the older ones—a not absolutely correct account. Spore-formation, he states, is due to the division of the protoplasm, so that a nucleus occupies the centre of each spore; these nuclei were already often visible, unstained, as clear spots in the dark granular

* Sitzungber. Niederrhein. Gesellsch. in Bonn, vol. 35.

sporangial plasma. The nucleus persists in the zoospore, and divides on its germination. He appears to have used very dilute hæmatoxylin for staining, and, as we shall see, his results are still valid. Strasburger, in the third edition of his "Zellbildung and Zelltheilung," gives (p. 56) a very imperfect account of the processes involved in spore-formation, and (p. 219) describes the nuclei of the hyphæ as ellipsoidal bodies with a nucleolus (Kernkörperchen). He saw nuclear divisions towards the ends of the hyphae, which he describes :—"Nur so viel ist in diesen äusserst kleinen Gebilde zu sehen, dass sie sich zunächst verlängern, dann an die Polen verdichtet erscheinen, dann auseinander weichen, den mittleren Theil fädenförmig erstreckend." I can hardly think that he really saw nuclear division at all. His technique, which he admits frequently fails to give good results, is to fix with absolute alcohol (chromic and osmic acids being unsuitable), and, after a prolonged stay therein, to stain the specimen with Beale's carmine, borax carmine, or hæmatoxylin; and if the last dye overstain, to decolorise with alum-water or a weak solution of hydrochloric acid. His figures (t. XIII. figs. 1–8) are drawn under a maximum power of 540 diameters, which is quite inadequate to show the structures visible in good preparations with a Zeiss J. (water imm. $\frac{1}{15}$"), which was, I believe, the power he used in examination.

M. Büsgen, in 1883, in his "Entwicklung der Phycomycetensporangien,"* gave an accurate account of the successive *appearances* presented by the protoplasm in the formation of the zoospores, but did not secure their correct interpretation. He failed to obtain uniformly good results by staining; but, by the use of glycerine mixed with dilute hæmatoxylin, he showed the presence of nuclei in Leptomitus, as lenticular bodies with a small nucleus, giving as nuclear reactions insolubility in artificial gastric juice, and swelling in sodium carbonate solution. He was unable, in the young sporange, to show definite relations between the nuclei and the origins of the zoospores; but, from their apparent juxtaposition in pairs after the homogeneous stage, inferred that bipartition took place during it.

Marshall Ward, a little later,† gave an independent confirmation to

* In Pringsheim's "Jarhbücher," vol. xiii.

† "Observations on Saprolegnieæ," in Quart. Journ. Micr. Sc. N.S. v. xxiii. (1887).

Büsgen's account of the zoosporange, with but slightly different interpretations of the facts. He did not stain, but inferred that bipartition took place before the homogeneous stage.

My own studies, which convinced me that Büsgen's interpretations were wrong, were put forth in a Paper "On the Formation and Liberation of the Zoospores in Saprolegnieæ," first communicated to the Association Française pour l'Avancement des Sciences at Nancy in August, 1886, and afterwards published (February, 1887) in the Quarterly Journal of Microscopical Science. I had not then succeeded in obtaining good stained preparations. Berthold, in his Protoplasmamechanik, published at the end of 1886, gave a welcome independent confirmation of my interpretation of the processes that occur in the sporange, but his study of the group left him still in doubt as to whether Büsgen's discordant results might not be correct for the species which the latter author had studied.

Ladislas Rothert, who again worked independently of myself and Berthold, gave a much more extended account of the formation of the zoospores in a Paper published in the Proceedings of the Cracow Academy towards the end of 1887, of which a German translation appeared in Cohn's "Beiträge" for 1888, under the title "Die Entwicklung der Sporangien bei der Saprolegnieen,"* with an appendix criticising my views on the liberation of the zoospores. He failed in staining the nuclei except in the vegetative hyphæ: the reagent used was iodine. This Paper I abstracted at length in the Annals of Botany,† with a running criticism on the matters in dispute between us, containing additional observations and arguments. I accepted his observations, however, as being absolutely exact so far as they went, a position from which I have in one important point receded in the present Paper.

Dangeard gives an account of this group in a Paper entitled "Recherches histologiques sur les Champignons";‡ and Professor Humphrey's admirable monograph of "the Saprolegniaceæ of the United States, with notes on

* This is the Paper which I refer to under Rothert's name.

† Vol. II., Aug. 1888—"Recent Researches on the Saprolegnieæ: a Critical Abstract of Rothert's Researches."

‡ Le Botaniste, sér. 2, 1890.

other species," published in the Transactions of the American Philosophical Society, 1892, deals with cytological questions. Both of these Papers were published after the greater part of the present Paper was completed.

This summary practically exhausts the literature of the cytology of the Saprolegnieæ if we defer, as is most convenient, the study of their so-called sexual reproduction, which has been the subject of a far more copious, if not a more satisfactory, treatment.*

SECTION 2.—Technique.

My study of the Saprolegnieæ had led me in 1887, to express the conviction that further advance in our knowledge of their spore-formation could only be obtained by such improved methods of microscopical technique as would display the nuclei clearly. For some time this idea lay almost dormant; and the occasional attempts I made to discover the path in this direction were so unsuccessful that I had almost given up trying, when Rothert's Paper started me afresh on the search, in order to clear up the many points which were still left doubtful. Soon after, in the autumn of 1888, I had the fortune to come on a method that could be trusted to give a uniformly good differential stain of the nuclei, with "nature-true portraits" of every structure to be made out on the living Mould, and which besides allowed of the transference of the specimens to highly refractive media, and hence of their satisfactory study with homogeneous immersion objectives of high aperture. To effect this, it was necessary to surmount three obstacles.

1. The fact that in this group the protoplasm teems with proteic granules ("microsomes") which take most nuclear dyes, and from their size and abundance mask the small vesicular nuclei.†

* It belongs to that form of paragamy which I have designated endokaryogamy. (Problems of Reproduction, p. 75.)

† This difficulty was felt by all my predecessors. Büsgen got only insignificant results. Rothert failed completely, finding that hæmatoxylin and carmine solutions stained the protoplasm in its whole mass intensely before the nuclei were more than slightly stained (p. 305). Strasburger's directions, in the "Botanische Practicum" (ed. 3, p. 56), suggest *three* ways of fixing, and *two* of staining, and says that thus we may easily (*leicht*) demonstrate numerous small nuclei. But the student who tries the six methods implied will probably fail in all, except perhaps by a lucky accident, which he will find it difficult or impossible to repeat at will.

2. The hyphæ are frequently wide with a large lumen and a thin parietal layer of protoplasm lining the delicate cell-wall; so that they collapse with extreme readiness on transference from one medium to another, a circumstance which frequently occurs when absolute alcohol is used as the "fixing" reagent. Hence, so far as I know, glycerine has been the most refractive medium hitherto utilised; and its refractive index (1·46) is too low to restore transparency to the stained sporangia.

3. A minor point is that the hyphæ, from their brittleness, kink and felt very readily, so as to obscure their true relations.

These difficulties I have stated at length, and shall refer incidentally to others that justify and explain what might else appear a disproportionate treatment of the dull minutiæ of the laboratory. Some interest may be added by the discussion in each case of the theoretical grounds on which the treatment adopted was founded. I may here note that my cultures were made on flies and meal-worms,* and embraced the following species: *Saprolegnia ferax* (vars. *monoica*, *monilifera*, and *torulosa*), *S. corcagiensis* (possibly a more slender form of *S. ferax*); *Achlya polyandra*, Hild., and *A. apiculata*, Dby.; *A. recurva*, Cornu; *Leptomitus lacteus*, and *Aphanomyces* sp.† The *S. torulosa* was from salmon disease, and was sent to me by my friend Mr. Allan P. Swan, of Bushmills, county Antrim, whose interesting studies on the biology of this form will, I trust, soon be published. The others have appeared, from time to time, in the tanks at the Queen's College, Cork, or were obtained from ponds and ditches in the neighbourhood of Cork.

(A.) *Selection of Specimens.*—The growth, if small enough, is merely floated on to a slide or cover; if large, the host is floated bodily on to a slide, and the growth expanded by a gentle stream from a wash-bottle. I then cut off as much as I want (with or without a fragment of the host) by pressure with the side of a needle, or with a dull scalpel; for any sharp edge leaves the cut ends patent, so that water enters and ruins the protoplasm, while the blunt instrument seals the hyphæ and leaves them turgescent. I frequently put a thin cover on the slide, and float the growth over it, so that

* I have used with advantage, latterly, the larvæ of Chironomus, preserved in spirits.

† I cannot identify this species, as it produced no oospores; it was saprophytic, with abundant intra-matrical hyphæ.

the selected patch is left on the cover, where it is kept through all subsequent processes. The host with the rest of the growth is drawn back and returned to the water, and as much liquid as possible drained off the specimen.

(B.) *Fixing and Hardening.*—Corrosive sublimate ($HgCl_2$), in saturated solution, commended itself for the following reasons:—1, it acts with extreme rapidity; 2, it gives no precipitation or false structure visible even under the highest powers; 3, it causes no irregular contraction, or collapse, or swelling, as absolute alcohol or glacial acetic acid are apt to do; 4, it does not interfere with staining like acid fixatives; 5, it determines no swelling like dilute acetic acid (10 per cent. and under). A drop or two is poured on the slide, taking care not to disturb the mycelium, which is then inverted over a watch-glass full of the solution, in which the specimen comes off; or, if the solution is on a cover, I put that directly in the watch-glass full of the solution. The watch-glass should be thick and with the bottom ground flat for steadiness, and contain one or two pieces of flat glass to prevent the cover touching the inside of the glass and so sealing off the liquid in the bottom. If possible the specimen should be kept on the cover during all the subsequent processes. A flat-bottomed capsule, like a small crystallising dish, is even better than a watch-glass.

In effecting all changes, I suck off the liquid with a pipette drawn to a very fine point. Care must be taken on no account to draw in any of the hyphæ of the specimen, for they would be irretrievably ruined. After most of the liquid is drawn off, the rest can be removed by draining; or the cover, with the specimen on it, can be removed and drained. The watch-glass must be covered with another or with a piece of plate-glass, except during the actual aspiration and replacement of liquid. The order of fixing and hardening is as follows:—Sublimate, water, 50 per cent. spirit, 90 per cent. spirit, absolute alcohol half an hour each.

(C.) *Staining.*—The chief modification from the Naples method of alcoholic borax carmine consists in the use of a strong alcoholic solution of glacial acetic or formic acid for differentiating; for I found it impossible with mineral acids to decolorise sufficiently the microsomes, and yet ensure a nuclear stain. Besides this, I have found it advisable to give a

slight prelminary stain with nigrosine (which I call a *mordant*), as the carmine is more distinctive after this treatment; and a subsequent *toning* with the same dye, to lessen the glare of carmine in the full light of the Abbe condenser.

The solutions I use are:—

1. Mayer's alcoholic borax carmine.

2. 50 per cent. spirit, 4 vols.; glacial acetic (or formic) acid, 1 vol.

3. In solution 2, enough nigrosine is dissolved to make the solution opaque in the body, transparent in the neck of a one-ounce stoppered bottle ('stock nigrosine solution').

(*a*) *Mordanting.*—The specimen is transferred to 50 per cent. spirit, to which a few drops of the 'stock nigrosine' is added. This is left for five minutes, or until the specimen is a *light* steel gray.

(*b*) *Staining.*—The mordant is replaced by the borax carmine, in which it stays for a variable time. As little as four hours suffices for small specimens; three days for the oangia. After this I wash by repeated changes of 30 per cent. spirit, until no more clouds of red diffuse out in the space of five minutes.

(*c*) *Toning* is effected by a stay in the stock nigrosine, until the specimen is distinctly purple instead of crimson. For oogonia I use only a few drops added to the differentiating solution.

(*d*) *Differentiation* is effected in the acid alcohol in 12–24 hours, for vegetative hyphæ and zoosporangia; for the oangia I prolong the bath to three days.

(D.) *Clearing and Mounting.*—(*a*) '*Clearing*' implies the replacement of the interstitial liquid of dead protoplasm by a medium sufficiently near the refractivity of the trama of the protoplasm to reduce notably its opacity, which is produced by its physical and consequent optical heterogeneity.*

* A. B. Lee was, I think, the first to explain clearing in this way, "Microtomist's Vademecum," 1885, p. 213, but he did not note the significance of the opacity as a *post-mortem* phenomenon, a point which I first indicated in a Paper "On the State in which Water exists in Living Protoplasm," communicated to the British Association (Newcastle, 1889), and read at the Royal Microscopical Society ("Journal," vol. x., 1890). I am at present engaged in the further elucidation of this matter, with the aid of instruments purchased by a grant from the Royal Irish Academy.

The transference to such a medium is apt to induce violent diffusion currents, and the consequent collapse of the hyphæ. To avoid this I make the change as gradual as possible, adding the clearing medium drop by drop at first, or even beginning with a solution of it in absolute alcohol. The intervals between successive additions should be at least five minutes to begin with; but afterwards we may go faster, and with larger quantities at a time, drawing off the excess of liquid occasionally, till practically all the alcohol is replaced. Then I change Abe's liquid for a fresh supply of the medium; and once again for precaution if I intend finally to mount in balsam.

(*b*) *Mounting in Balsam.*—In this case the clearing medium will be carbolised xylol, 4–6 vols.; crystallised phenol, 1 vol. The phenol is introduced to meet a difficulty which most mounters in balsam have experienced: the rapid evaporation of the mixture of xylol and alcohol, while the hygroscopicity of the latter induces the precipitation of water-vapour from the atmosphere in droplets, which adhere to the specimen and ruin it. Now, we know that anhydrous phenol, though taking up a notable quantity of water, forms a hydrate which is still freely miscible with benzole* and with what we may term 'ethereal' liquids generally, to form limpid media that dissolve balsam; and my anticipation that its use would meet the above difficulty has been fully justified. It is for a similar reason that the oils of cloves, bergamot, cassia, and sandal-wood are preferred by the histologist to the majority of essential oils.†

The addition of balsam frequently induces collapse. I find this best avoided by putting largish fragments of dried resin of Canada-balsam into the watch-glass, as far as possible away from the specimen, and leaving

* See Lee, *op. cit.*, p. 216. I may here note that the above oils, *if free from phenol*, may be restored readily from their residues contaminated with alcohol and water, by the following treatment:—Shaking with two volumes of water removes the alcohol. After decantation, and filtration through a filter first dried by heat and then moistened with turpentine, dehydration is completed by letting the oil stand over fused calcium chloride, or stove-dried sulphate of copper. A second filtration completes the operation. I may note that standing over a few fragments of calcium chloride will soon clear cloudy balsam or dammar.

† I found the phenol insoluble in commercial "benzoline," which is only a mixture of light paraffin oils belonging to the marsh gas and olefine series.

it undisturbed for forty-eight hours, by which time solution and diffusion are completed. The specimen may then be lifted out on its thin glass cover, if prepared on one; if not, one must be cautiously slipped under it. This is placed on a slide, and covered by another selected of sufficient thinness for the wide-angled objective; and the preparation is dried at a gentle heat, fresh rather thick xylol-balsam being added from time to time to compensate for the loss of liquid.

If any felting has taken place it may often be remedied before mounting by gentle agitation in the watch-glass, aided by judicious combing (not teasing) in the medium with a fine and thoroughly bright sewing-needle.

(*c*) *Mounting in Essential Oil.*—I found it impossible to ensure perfect and uniform success, in transferring the oogonia to balsam, since these structures are only slowly permeable, and very liable to collapse. I hence resolved to mount direct in a suitable clearing medium, and use oil of yellow sandal-wood,* with a little crystallised phenol added, and enough oil of cassia or cinnamic aldehyde to raise the refractive index to 1·56.

The substitution is effected just as with the carbolised xylol. As the normal arrangement of the hyphæ is irrelevant to the study of the oogonia I usually fix, stain, and clear the culture *en masse* (often host and all), conducting the operations in wide-mouthed corked bottles, and with far more ease than in the watch-glass. Owing to the much greater volatility of alcohol than the essential oils, I often complete the process by exposure in a watch-glass over H_2SO_4 in an exsiccator. Bits of the felted mass may then be taken out, teased in a watch-glass full of the medium, and lifted out on a cover which can be reversed on to the slide, or put face up and covered by another of proper thinness; or else I tease a fragment on the slide, and cover without further ado.

(*d*) *Glycerine Medium.*—If it is desired to leave the fatty matters *in situ* the above methods are inapplicable, and absolute alcohol must be avoided; after staining, &c., and careful washing with dilute alcohol to remove acid, the specimen is transferred to the saturated solution of sulpho-carbolate of zinc in glycerine (made by boiling equal weights together), until solution

* Recommended by Neelson and Schiefferdecker, according to Lee, *op. cit.*, p. 220. Its freedom from colour is its great advantage over oil of cloves or cassia, and carbolic acid.

is perfect. This liquid has about the same refractivity as crown-glass, and has been recommended for homogeneous immersion. It gives good pictures; but I have not mastered the conditions under which the stain is permanent, and the formation of crystals is avoided; hence I do not regard the method as a fully developed one. Transference is effected by using a very dilute solution in water, and concentrating slowly by evaporation in the air, or by letting a drop at the side of the cover run in as the spirit evaporates. Collapse is less ready than with 'ethereal' media.

(*e*) *Sealing.*—Both these liquid media are readily sealed, *without any need of cleaning slide or cover*, by Kaye's coaguline applied hot with a camel-hair brush. Next morning the cement is set, and must be pierced with a needle if raised by blobs of the medium. The slide is then washed with a few drops of spirit, and a fresh ring of coaguline painted over the first; owing to its brittleness when hard, a final coat of gold size is advisable, but if the gold size be applied too soon the water of the cement may diffuse into the oil, and ruin the specimen. My sandal-wood preparations, after standing for five years, are as good as on the first day, and at least equal to the balsam ones; and I shall probably rely in future on the former only.

(E.) *Quick methods.*—1. A rapid mode of preparation which usually gives first-rate nuclear preparations is to treat the specimen with stock nigrosine for five minutes; wash with glacial acetic acid, cover, and let glycerine run in from the edge. If, as a preliminary, the specimen has been fixed with sublimate and washed, the object is usually very satisfactory; but if used directly the microsomes swell, yet even so the nuclei are often exquisitely shown.

2. To demonstrate the fat globules, tincture of alkanet is used as well as the nigrosine (best *after* the latter), and glycerine run in.

I have not tested either of these methods for permanence. By using any of the above methods we can ensure successful and nearly uniform preparations; and manipulative skill and caution are only required to prevent felting, spilling, or drying up of the specimens.*

* I have not tried section-cutting, since the above methods proved satisfactory; and the inspection of sections made by my friend, Mr. Harold Wager, whose skill in these matters is exceptional, convinced us both that the results to be obtained were inferior.

Section 3.—The Habit of the Hyphæ.

The majority of the species of Saprolegnieæ I have examined send numerous short, slender "intramatrical hyphæ," or rhizoids into the substratum, and long "extramatrical hyphæ" into the water, each of which normally terminates in a "primary zoosporange," as we shall see below. From the base of these primary hyphæ lateral branches or stolons are given off, which run along outside the substratum, giving off rhizoids on the one side, and extramatrical hyphæ similar to the primary ones on the other. After the formation of the primary sporanges branching takes place, with sporanges at the end of each branch according to one or other of the schemes which we shall describe below; but finally in old cultures the branching becomes irregular, and the twigs run out into fine, tapering filaments as if in search of "fresh fields and pastures new." This type is of course that suited to the types that grow on solid nutritive substrata, such as dead animals, &c.

Leptomitus lacteus on the contrary sends out lateral branches freely from the floating hyphæ; they come off from near the apex at an acute angle, and grow freely so as to form false dichotomies, while the frequent constrictions of the hyphæ enable them to float very freely, bending rather than breaking at a touch. I believe this to be an adaptation to the peculiar life-conditions of the species, which grows attached to inorganic substrata in liquids rich in organic matter such as distillery wash, though it retains the habit when grown in clean water on an organic substratum. In *Saprolegnia ferax* grown on cress seedlings, which become pulpy so that the medium becomes charged with food materials, we may constantly observe the same false dichotomies.

All the floating hyphæ are at first slender, full of plasma with vacuoles few and far between; the rhizoids usually have only a parietal layer of protoplasm, with few granules, occasionally thickened into a plug across the lumen. But the former grow in width as well as in length; they are strongly turgescent, and show every transition from their early form to large tubes, with a thin parietal investment of protoplasm, and a gigantic lumen frequently traversed by a network of cords of protoplasm, which show the movements of streaming, fusion, and separation, which have been known to and described by the earliest observers.

SECTION 4.—The Cytoplasm and Cell-sap.

The protoplasm in the larger hyphæ of Saprolegnia consists of a delicate outer layer of *hyaloplasma* containing minute oil-drops, which passes by continuity into an inner layer rich in highly refractive granules or microsomes. This granular protoplasm is prolonged into a network of ridges, projecting inwards and becoming detached to form the threads just mentioned: these ridges everywhere run by continuity into the parietal hyaloplasm, and between the microsomes consist itself of hyaloplasm. I cannot believe that there is any essential distinction between the two forms of plasma; for it is a character of viscid substances, as shown by physicists, that any foreign bodies contained therein tend to leave a superficial layer clear from them and homogenous. However, the ridges and threads take a deeper stain than the outermost layer. Towards the growing points, whether of extra- or intramatrical hyphæ, the extremity is always, as it were, plugged by hyaloplasm: this has the same refractive index as the cell-wall in its living condition of imbibition, for the outline of the end of the hyphæ is single in optical section. This apical hyaloplasma is free from refractive microsomes, whether proteid granules or oil-drops, but contains what, under the highest powers available, appear to be minute droplets. Treated with iodine the droplets appear as dark granules; in stained balsam specimens they are lighter coloured and less refractive than the protoplasmic matrix in which they lie. Hence they are probably drops of a solution of glycogen, which would of course be extracted in the fixing processes, leaving clear spaces. The appearance of a reticulate structure in the hyphal granular protoplasm is, I am convinced, a fallacious one, the threads in the living hyphæ lying on the inner side, and not in the substance of the parietal investment, and in this respect I am borne out by Schwarz.* Since the appearance of Bütschli's work on "Foam Structures" I have carefully sought for these in the clear protoplasm, and I have been unable to convince myself of their existence in undamaged hyphæ. But wherever the hypha is damaged, the swelling and solution of the microsomes

* "Studien über Protoplasma," in Cohn's "Beitrage," vol. v.

gives rise to an exquisite foam structure with a fine alveolar layer at the surface, the nuclei at the same time approximating in groups (t. xxviii., fig. 1).

The streams of granular protoplasm are chiefly longitudinal, but frequently show a certain tendency to assume an elongated spiral arrangement, so as to present a graceful trellis-work under powers low enough to display both sides of the hypha with the same focus. The general direction of the streams is acropetal, but reverse currents are frequent. In hyphæ cut off by pressure with the side of a needle, the currents constantly vary in direction. Many authors have written as if this streaming were the sole cause of the accumulation of protoplasm at the ends of the hyphæ, and especially in the sporange; but closer observation will show that the amount of actual transference is quite inadequate, and that in every part of the hypha the protoplasm is actually growing in substance, presumably from the stores of food dissolved in the cell-sap. This food cannot however exist in the form of dissolved proteids; since $HgCl_2$ gives no precipitate in the cell-sap, nor do we obtain the biuret reaction with cupric sulphate and caustic alkali. In the more slender hyphæ with sparse vacuoles the currents are not obvious.

In the extremities of hyphæ that have ceased elongating, the plug of hyaloplasma is absent, so that the apex has in this respect the same character as the sides.

Section 5.—The Microsomes.

(*a*) *Protein Granules.*—These are found everywhere in the protoplasm; they are highly refractive and of irregular form; the largest may exceed 1 μ in diameter. They swell up in acetic acid (1 per cent. or glacial), but not after treatment with the acid alcoholic solution of nigrosine, which fixes them. I believe that they are nearly solid, like the aleurone grains of higher plants. They are few and scattered in the rhizoids, more numerous in the elongating hyphæ, most abundant in the protoplasmic accumulations of the zoosporanges and oangia before homogeneity. They take nuclear stains readily, and give them up to acids only less reluctantly than the chromatin mass of the nuclei.

(*b*) *Fat Granules.*—These deserve a special mention. They are best shown by the method with alkanet and nigrosine in uninjured hyphæ. They are minute globules, varying in size from the smallest particles visible to about 4 or 5 μ in diameter. They are everywhere found in the granular protoplasm, and some of the finer ones lie in the parietal plasma external to the others and the microsomes, doubtless owing to physical causes. If *before fixing* the protoplasm has been acted upon by water, or crushed, we find that not only are larger oil-drops formed by the coalescence of the others, but the fatty contents are more abundant, as if the protoplasm had undergone catalysis into fat and a derived substance. It will be remembered that Loew* found that, after treatment with alkalies, plastin gives the albumen reactions with ferrocyanide of potassium, which it does not do before; and concludes that, on this account, plastin is an albuminoid matter contaminated by fats, etc., which mask its proper reactions. Our observation indicates a closer union of the non-nitrogenised matter and the proteid in the living plasma; and confirms Reinke's view that plastin is a distinct chemical species.

(*c*) *Glycogen Droplets.*—These are recognisable as minute refractive drops, which stain a deep mahogany brown with watery solution of iodine. Owing to their minute size and high refractivity, their colour is only well shown in the full aperture of the Abbe condensor.

SECTION 6.—Cellulin Bodies.

Pringsheim gave the first account of these bodies,† which are especially conspicuous in Leptomitus, where they were first regarded as nuclei. The following account is abstracted from Pringsheim's careful description. When young they are usually flattened disks or polyhedra with rounded angles, but become spheroidal as they grow, and in all cases show, when fully developed, a concentric lamination comparable with that of starch grains (but much coarser); this lamination and a spherical form are observ-

* "Botanische Zeitung," 1873.

† "Ueber Cellulinkörner, eine Modification der Cellulose in Körnerform," in Berichte d. Deutsch. Bot. Gesellsch. I. (1883), p. 288.

able from the first in Leptomitus and Achyla. In Apodya and Saprolegnia they are frequently collected in groups, touching by their flat sides. The small granules may be formed free in the cytoplasm or budded off larger ones. They have, in the fresh state, a dull grayish blue lustre, indicating a slightly higher refractivity than the cell-sap, due to the fact that they are imbibed with the cell-sap. [As they become almost invisible in balsam, the refractive index of the solid substance must be between 1·51 and 1·52.]

The chemical reactions are as follows, according to Pringsheim:—

1. In aqueous and alcoholic solutions of iodine they do not stain, but have, through imbibition, the same tint as the solution.

2. They are insoluble in the solvents of fat and resins, [and take no colour from tincture of alkanet].

3. They give none of the colour reactions for proteid, and take up even less of the ordinary colouring-matters than the cellulose walls of the hyphæ.

4. Caustic alkalies in the cold do not affect them even by prolonged action; boiling solutions, however, *after the first few minutes*, make them pale.

5. Concentrated hydrochloric and nitric acids have no effect in the cold. Boiling for a short time in HCl or Schultze's mixture has no effect. Prolonged boiling in Schultze's mixture renders them pale and inconspicuous.

6. They dissolve more readily than the cell-walls in sulphuric acid diluted with an equal part of water, and leave no residue.

7. In the same way they dissolve in a moderately strong solution of chloride of zinc.

8. They do not dissolve in ammoniacal solution of cupric oxide.

On these grounds Pringsheim identifies them with a form of cellulose closely allied to Frémy's fibrine and to fungus-cellulose, and perhaps even nearer to starch-cellulose.

Pringsheim has described* a 'periplasm' in the oangia of *Achlya colorata*

* "Neue Beobachtungen üb. d. Befruchtungsakt von Achlya u. Saprolegnia, in Sitzungsb. d. Akad. d. Wiss. Berlin" (1882).

which is resolved into cellulin granules. I have not examined this species, but in the objects of my study the cellulin granules disappear in both zoosporange and oangium on their delimitation into spore origins. Rothert has shown their function in the formation of the basal septum. In the moniliform genera, Leptomitus and Apodya, the blocking of a constriction by a cellulin granule, which fuses as it were with the walls, may effect septation.

Pringsheim notes that the Chytridian endoparasites of Saprolegnieæ do not attack the cellulin granules; I may say the same of the Monadine parasites.*

SECTION 7.—**The Nuclei.**

Distribution and Form.—The nuclei, as might be expected on physical grounds alone, are confined to the granular plasma, leaving the Hautschicht free; and never wandering into the apical hyaloplasmic plug of growing hyphæ. In the nearly solid younger hyphæ they are spherical or nearly so, and have a maximum diameter of 3μ. In the moving streams of the walls and intravacuolar strands of older hyphæ they are irregularly fusiform, not lenticular as frequently stated. I had previously, on *à priori* grounds, attributed this change of form to the plasticity of the nucleus; and I have since ascertained the truth of this conjecture, by observing that in the living hyphæ a nucleus lying in a strand of motile plasma elongates as the stream became more active, and shortens when it slackened.†

Resting Stage.—The nucleus in the zoosporange, the antheridium, and the young nearly solid hyphæ is approximately spherical, with a distinct wall, a peripheral layer of hyaloplasm staining less deeply than the other two constituents, and a central spheroidal mass of nuclein or chromatin which I intentionally refrain from calling a 'nucleolus.' This vesicular form of nucleus is characteristic of most Protophytes, of the Monadina and Myxomycetes, of most‡ Protozoa (excluding the Ciliata, the Suctoria, and a

* It is impossible, from the above considerations, that any trained observer could have taken cellulin granules for parasitic amœboids, an accusation which Pringsheim makes against Zopf, as we shall see in the discussion on the formation of oospores.

† Observed with Zeiss J. ($\frac{1}{16}$ water imm.).

‡ Zopf ("Schleimpilze," p. 19) limits the term 'nucleus' to what I call the nuclear mass; for he speaks of the "Kern von hellem Plasmahofe umgeben."

few Lobosa), the Hydrozoa, and of the young ovarian ova of most other animals.

Active State.—In the larger hyphæ the nucleus is seen in the "active" state; its structure here is nearly everywhere the same in essentials: a nuclear wall; a vacuolated hyaloplasma; and a nuclein mass which is frequently excentric, especially in the much elongated nuclei of wide hyphæ with rapidly streaming protoplasm. In glycerine specimens prepared by the quick method, the vacuolate hyaloplasma is well shown, and may be regarded as forming a peripheral layer lining the nuclear wall, a central layer investing the nuclein mass, and connecting threads suspending the latter. The nuclein mass, which is spherical in the resting nucleus, is very irregular in the active state and might almost be called amœboid; movements of the "nucleolus" have been repeatedly recorded by other observers,* but they are probably passive and due to the movements of the nucleo-hyalo-plasma, which, for brevity, we may call by Schwartz's term adopted by Strasburger, *linin*. In these movements the nuclein may be divided into two or more unequal parts. In the pale nuclei of rapidly elongating wide hyphæ, the nuclein is very much reduced in size, and the linin is abundant, showing a minute stippling probably due to "foam structure." This seems to have no relation to division.

The 'active state' may be studied in the life in wide hyphæ where the microsomes are abnormally few. Such hyphæ occur frequently among cover-glass preparations of cuttings; they may be due to the presence of endo-parasitic amœboids (of *Pseudospora? Lindstedtii*) or simply to starvation, for I have also repeatedly seen such clear filaments without being able to detect the presence of amœboids, and where there existed none of the loose particles dancing in the cell-sap which early betray the existence of the former. Under these circumstances I have seen the living nuclei in Achlya and in Leptomitus lacteus under as low a power as Zeiss D ($\frac{1}{6}$), but found the J ($\frac{1}{15}$ water imm.) necessary for their study. Rothert's account of these nuclei, as seen under these conditions, is very accurate:—"Dagegen sind in vegeta-

* In the germinal spot of Blatta by Brandt (in Schulze's "Arch. f. Mikr. Anat." vol. x., p. 305); in that of *Silurus glanis*, by Eimer (*ibid.*, vol. xi., p. 325); and figured and described by Zopf in *Vampyrella pendula*, &c. ("Schleimpilze," p. 16, and fig. 3, vi.).

tiven Fäden mit zartem Plasmawandbeleg die Kerne auch ohne Tinction meist unschwer zu sehen. Sie erscheinen als *rundliche* oder spindelförmige Gebilde von mattem Aussehen; nur die Peripherie (= nuclear wall) und ein glanzendes Korn im Centrum (= nuclein mass) von der Grösse gewohnlicher Plasmakörnchen (= microsomes) treten deutlich hervor; der Raum zwischen beiden (= hyaloplasma + nuclear sap) sieht wie substanzleer aus, doch werden durch Iod die fragliche Gebilde in ihrer ganzen Masse braun gefärbt" (p. 305 Anm.). It will be noted that he correctly describes the form as spheroidal or fusiform, not lenticular.

Nuclear Multiplication.—Multiplication of the nuclei is of rare occurrence in the greater part of the area of the wider hyphæ, where alone they can be seen well enough for study: in these, dividing nuclei occur grouped in zones. But at the base of these wide hyphæ we may frequently find dividing nuclei, sufficiently abundant to touch by their peripheries. It would seem indeed that the immense majority of the nuclei are formed here by bipartition, to be carried away to the ends of the hyphæ by the plasmatic currents; and that they become more and more widely separated as they advance, through the interstitial growth of the protoplasm, the latter fact being most obvious in the formation of the zoosporange.

The best objects for the study of the nuclear divisions are the little isolated tufts of a three or four day old culture on a mealworm punctured in several places. These are readily washed out by rough handling in filling up the glass after pouring away the fouled water, *e.g.* by letting a strong stream come from the tap. Such tufts may be lifted out on a cover and prepared by the "quick method" after washing from a wash bottle, and gentle teasing if necessary. They show beautifully the haustoria and basal branching of the hyphæ. As the size of the nuclei is at most 5 μ by 3 μ, and the nuclein mass barely exceeds 1 μ in diameter, it is obvious that our examination of details is soon arrested by the optical limitations of our instruments, which can hardly separate details less than 0·2 μ. But even so, the appearances are sufficient to reveal that the division of the nuclei is essentially a special case of karyokinesis, intermediate between direct division and the commoner processes. We find nuclei with the nuclein disposed in a transverse plate, and focussing shows this to be composed of

four minute granules; further on we find these equatorial plates composed of eight granules. From the fact of the divisions always taking place in a transverse plane, it is difficult to make out the true form of these granules; but in one case where the equatorial plate was oblique I have seen them elongated in the equatorial plane. The next stage is that which I mentioned in the Comptes-rendus of the French Academy, that the nuclein masses form two crescents back to back, each showing a dim striation as if made up of several short parallel filaments. We next have the granules in two groups of four each, in a more elongated nucleus; and finally the original nucleus by a median constriction gives rise to two new ones, and the process is completed. (Pl. XXVIII. figs. 18, 20; Pl. XXIX. figs. 23, 24.)

Interpreting the process by comparison with larger forms we may describe it as follows, using the terminology of Strasburger's work, "Kern- und Zelltheilung," 1888:—I. *Prophasis.*—The nuclein mass separates into four short rods, which become grouped into the nuclear plate. Here they undergo longitudinal division, so that the plate consists ultimately of eight rods. II. *Metaphasis.*—The eight rods resolve themselves into two crescentic groups of four each, which separate from one another. III. *Anaphasis.*—The four rods in each group diverge to form a lozenge-shaped tetrad, and ultimately approximate to form a single nuclein mass; the nuclear wall between the two nuclein masses is constricted, and so the nucleus is finally divided into the two daughter nuclei."

The majority of vesicular nuclei divide in this way by constriction, without any solution or disappearance of the nuclear wall. Strasburger puts forth the probable explanation* that the presence of a quantity of intranuclear plasma renders the penetration of cytoplasm from without unnecessary, and he correlates the permanence of the nuclear wall in Protozoa—"und vielleicht auch bei den Siphoneen und *eventuell auch bei vielkernigen Algen und Pilzen*"—with the fact that here nuclear and cell-division are independent.

I may add that Zopf figures† (after Brass) a stage in the Monadine

* "Kern- und Zelltheilung," pp. 223, 224.

† *Op. cit.*, fig. 4, IV*c*.

Pseudosporidium brassianum, which obviously corresponds to my "double crescent."

SECTION 8.—The Zoosporange.

1. *Distribution.*—The zoosporange is almost always terminal in Saprolegnieæ, intercalar ones being formed only exceptionally in old and irregular cultures. As for a long time each hypha ends distally in a sporange, we have 'infructescences' formed (if I may be permitted to coin an equivalent for the German 'Fruchstand'), which are highly characteristic of the genera and species. In Achlya, Aphanomyces, and Dictyuchus the secondary sporanges are in sympodia; for after the primary sporange is walled off the supporting hypha grows out immediately below into an

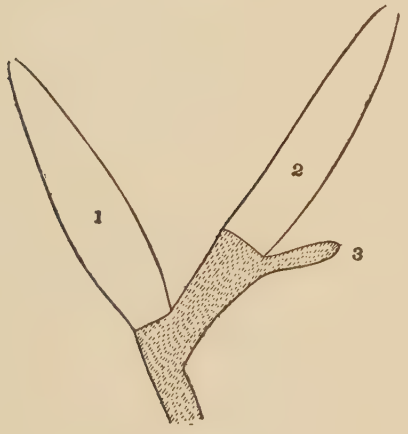

FIG. A.
Sympodium of Sporanges of Achlya.
1, 2. Discharged Sporanges.
3. Young Innovation.

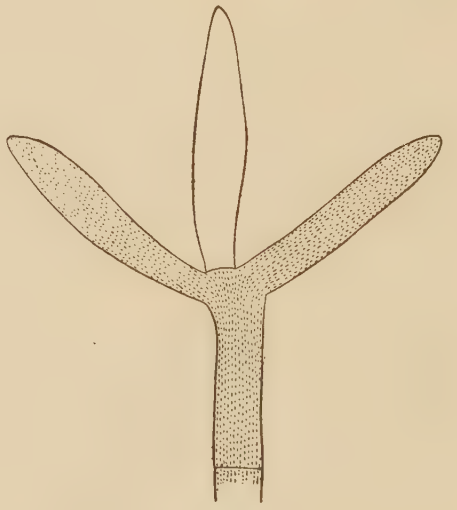

FIG. B.
Infructescence of Achlya, with double innovation.

innovation, or sometimes two or three. These usually extend beyond the primary sporange before being limited by the formation of other sporanges, the result being the production of long sympodia, as in the wood-cut (fig. A): when two or more innovations arise simultaneously, their septum may be formed lower down on the hypha that bears them, and so bifid or trifid sporanges are formed (fig. B).

In *Leptomitus lacteus* the formation of sympodia is in principle the same,

but the lateral branches are formed before any sporangial differentiation takes place; and the whole of each hypha that ends in a sporange is ultimately converted into sporangea in basipetal succession down to its connexion with the sympodium to which it belongs (fig. c).

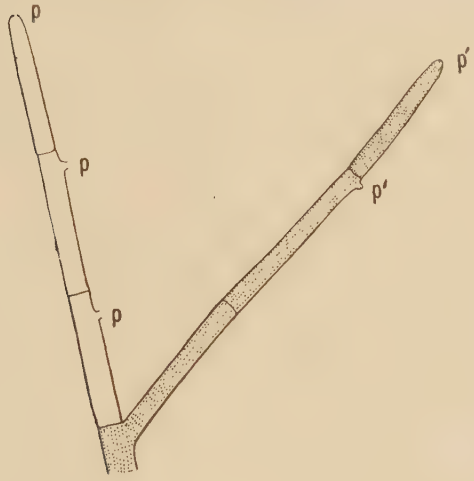

Fig. C.
Sporanges of *Leptometus lacteus*: p = discharging process after opening; p', before opening.

In the majority of the species of Saprolegnia proper the innovation takes place by the supporting part of the primary hypha, growing through the emptied terminal sporange, and forming a new sporange, more or less within the old one, or, if the growth be strong enough, beyond it. Basipetal sporangial differentiation of the supporting hyphæ is not infrequent here, especially in the form *S. torulosa*, but not universal as in *Leptomitus lacteus*.

2. *Formation and Closure.*—The formation of a sporange is due to the accumulation of the protoplasm in the hypha, which frequently enlarges so as to become club-shaped; in Achlya and Leptomitus the enlargement is usually slight. This accumulation may be seen towards the apex of wide-lumened hyphæ to begin by the parietal investment rising into spheroidal hillocks over the nuclei, which are here no closer than lower down, clearly showing that the process is due to *growth*, and not to *influx*, of the plasma.* This process is accompanied by the bodily onward movement of plasma and nuclei towards the apex, so that the lumen is frequently obliterated completely, though it may persist in 'starved' sporanges as a wide lumen, or in normal sporanges as a single vacuole or several ovoid spaces. I quote the account given by Rothert as abstracted by myself.

"At first the thick protoplasm of the sporangial part of the hypha passes gradually into the thinner investment of the basal part; but soon the

* Compare the small proportion of protoplasm to nuclei in figs. 2, 3, 4, 86, with the great increase of protoplasm in figs. 5, 7, 8, 15.

contrast is sudden and sharp. Then at this junction the granules" and with them the nuclei "disappear or migrate from the protoplasm, so as to form a longish ring of hyaloplasm, which grows at its inner circumference to finally form a transverse disk, extending across the hypha from wall to wall, sharply bounded towards the basal hypha, but on the sporangial side gradually merging into the granular protoplasm.

"In about half a minute the transverse septum appears (simultaneously) at the base of the disk, at first pale ('vervaschen'), soon sharply contoured.

"In favourable cases we may see that a rather broad basal section of the disk of hyaloplasm assumes a higher refraction; this gradually thins off, and becomes more clearly defined, and finally condenses ('sich verdichtet') into the septum, which is clearly not formed from a granular cell-plate as Strasburger states. Before this, however, a number of Pringsheim's cellulin corpuscles had accumulated about the limited area, and fell by the appearance of the hyaloplasma disk into an upper and a lower group. As the upper group disappear on the completion of the hyaloplasmic disk, Rothert thinks it probable that the granules, consisting of a very soluble form of cellulose, are absorbed into the disk, and by their solution afford materials for the septum.

"Slight modifications occur in the formation of the hyaloplasma disk, according to the relative fulness or emptiness of the sporange."

At the moment of the formation of the basal septum in *Dictyuchus clavatus* Rothert has noticed a slight escape of cell-sap as evinced by the swarming of bacteria to the neighbourhood of the basal septum, accompanied by a diminution in the turgescence of the sporange at this stage, and by the shortening of the sporange in Saprolegnia at the same stage which he holds to indicate a momentary breach of continuity in the protoplasmic investment, a view that we may well accept.

After the formation of the septum is completed, "In the full sporange granules gradually wander into the hyaloplasmic disk, which thus becomes indistinguishable. In the other forms the granular protoplasm first retracts from the disk, with which it is only connected by a thin hyaline layer, investing the wall of a few delicate plasmatic threads, so that the lumen is

widest at the base of the sporange. Vacuoles appear then in the disk, soon enlarging, and communicating with the main vacuole of the sporange. The disk then thins in the centre, and rises peripherally up the walls. The granular protoplasm again stretches down towards the septum, and finally, by the wandering of the granules into the hyaloplasma, the latter loses its character."

In this way the sporange is differentiated from its supporting hypha, which in Saprolegnia frequently shows already signs of differentiation into a younger sporange.

Discharging Process.—The sporange, so constituted, is completed by the formation of the short tubular *process* (except in Aphanomyces, where no distinct process is formed), through which the spores are destined to escape, such a process is frequently if not always present in the allied order Chytridieæ, but not in the Monadineæ. The time of formation of the process varies greatly ; it may be formed even before the basal septum or at a variable stage in the differentiation of the spore origins. In Achyla, it is frequently only a slight modification of the apex of the sporange ; but in this genus, as in the others, it may be formed as an outgrowth of any part of the sporangial wall, except of course the basal septum. As in all other cases of apical growth the growing plasma is hyaline, and appears to bulge out the cell-wall.

"The bulging of the membrane continues with the accumulation of hyaloplasma until a short cylinder, with a nearly hemispherical top, is formed, filled with this substance. The convex terminal wall or 'cap' is duller and less sharp-contoured than the rest of the sporangial wall ; its boundary not being distinct from the protoplasm on its inner side. The hyaloplasmic plug soon becomes granular, except a thin layer lining the cap of the process."* The changes of character occur also in the terminal wall and end of the sporange of Aphanomyces, which forms no distinct process. The hyaloplasma concerned in the formation of the basal septum and the process agrees with the accumulations of hyaloplasma at the apex of the growing hyphæ, in

* Haberlandt ("Ueber die Bezieh. zwisch. Function u. Lage des Zellkerns, in Berichte der Deutsch. Bot. Gesellsch.," vol. v. (1887)) noted that a nucleus moves up to the point where a branch is given off. I have no observations on this matter; but it is abundantly clear that the protoplasm alone is concerned with the formation of the process, as with that of the basal septum.

containing the minute structures which we regard as glycogen droplets, and in containing no nuclei. In the latter respect the Saprolegnieæ differ remarkably from *Woronina polycystis*, the only Chytridian that I have examined, the process of which is occupied by a mass of deep-stained plasma with a nucleus, separated by a clearer zone from the mass of spore-origins in the body of the sporange.

The hyaloplasm of the process soon becomes vacuolated, so as to leave only a parietal layer, thickened into a cap towards the apex, and connected with the general protoplasm of the sporange by threads which may become granular (Pl. XXVIII. figs. 7, 13).

The Contents of the young Sporange.—In its younger stage, the sporange often shows obliquely transverse striation in such a manner as to appear produced by the packing or ramming in of successive masses of plasma which retain a certain amount of distinctness; this is indeed the probable cause of the appearance, which has nothing to do with the later segregation into spore origins; for it soon gives place to a much more complete homogeneity. Except for the thin parietal hyaline layer, the protoplasm is full of microsomes, and consequently very dark and opaque in the larger sporanges, milk-white by reflected light, dark-brown in a strong transmitted light. "We can distinguish between—(1) 'full sporanges' which at first contain no central cavity, to which class usually belong the smaller sized ones; (2) 'ordinary,' or 'normal' sporanges, with a fairly thick parietal investment surrounding the cell cavity or lumen (often two or three vacuoles in Achyla); and (3) 'starved' or 'poor' sporanges, with only a thin parietal investment, and an immense vacuole." Leptomitus and Aphanomyces only present "full" sporanges.

Nuclei are visible in the living sporange at this stage under quite low powers (Zeiss $\frac{2}{5}''$), as clear vacuoloid spaces obviously less refractive than the protoplasm: under intermediate powers they disappear, but on focussing the more superficial layers of even the coarsest sporangia with a J ($\frac{1}{15}$ water imm.) the nuclein mass can be seen with the clear areola around it. Rothert saw and interpreted this appearance correctly, for he saw "ründliche Körnerfreie hellere Stelle ziemlich regelmässig in der aüsseren Schichte des Protoplasmas vertheilt: in *ihrem Innern* sind mit grosser Wahrscheinlich-

keit die Kerne zu vermuthen; mitunter gelingt es sogar in einigen derselben bei ein gewissen schiefen Lager je einen matten sehr undeutlich contourirten Körper zu erkennen." It is obvious that, like Zopf, he here regards the nuclein-mass as the nucleus itself, an interpretation which, as noted above, he did not adopt in his note on the vegetative nuclei.

The structure of these resting nuclei has been already described (*ante*, p. 664). The part they play in subsequent processes has been much misunderstood; for Schmitz and Strasburger are both of opinion that their division precedes spore-formation; Büsgen and De Bary refer their division to a later stage. Rothert says correctly that they remain stable, and persist till the final repartition of the spores, but could not demonstrate this absolutely nor trace them through all the stages for want of good stained specimens; and he describes them only as *vermüthliche* nuclei. My specimens have brought the required demonstration by showing the nuclei unchanged from beginning to end.

The Differentiation of the Zoospores.—The formation of the zoospores consists essentially in the segregation of the apocytial protoplasm into unicellular zoospores, just as it does in the Siphoneæ (excluding Vaucheria) and Siphonocladaceæ, and also in Cladophoraceæ, the multinuclear chambers of which cannot be regarded as true cells, but present an intermediate condition between the cellular and the continuous hypha. It may be resolved into the following process:—1, the *accumulation and growth of the protoplasm round the nuclei* which begins even before the closing of the sporange from the hypha, and has already been discussed; 2, in the *concentration of the protoplasm* with excretion of liquid into a network of splits, which delimit incompletely the spore-origins; 3, the *gradual separation of these last in two successive* stages, a *preliminary* and a *final*, the two being interrupted by a temporary fusion of the origins into apparent *homogeneity*; the final *fashioning of the zoospores* with their flagella and segregation. The concentration of the protoplasm and segregation of the zoospores proceed as follows:—

"In normal sporanges appear numerous splits in the protoplasmic investment, stopping just short of the cell-wall, and opening into the vacuole;

these appear and disappear, and finally become constant, forming a honeycomb network. At first, numerous plasmatic bridges connect the origins so mapped out; but most of these soon disappear; this is especially clear in Achlya. Some protoplasm remains long distinct from the origins, apart from the continuous wall," this last always remains free from granules (Pl. XXVIII. figs. 5, 6, 7, 15).

In full sporanges the appearance of a zigzag slit indicates the segregation of the origin of the smaller sporangia (Pl. XXVIII., fig. 8, lower part); in the larger segregation is produced by the appearance of linear lacunæ (Spalten) which form a connected system.

In poor sporanges the segregation rather takes place by the aggregation of protoplasm in heaps, partly by its own growth, partly at the expense and by the thinning of the intervening part of the parietal layer. Here also plasmatic bridges may occur, and some fragments of protoplasm are often left out of the schema.

During all this time more or less of the protoplasmic streaming goes on; microsomes pass to and fro along the plasmatic threads, and slow movements of rotation affect the whole of the protoplasm in the sporange, so that the network of lacunæ shifts its position.

In Aphanomyces the first stage of segregation is due to the appearance of elongated vacuoles, which determine the concentration of the protoplasm into ovoid patches. These shift, enlarge, and finally form oblong cylinders, separated from one another by cylindrical vacuoles, which may be squarish or transversely elongated in optical section. The oblong origins, as we may now term them, are also seen to be parted from the parietal layer of protoplasm by vacuoles, on one side or embracing the origin like a zone, while numerous plasmatic threads extend lengthwise to bridge over the cylindrical vacuoles between the origins, and radially to connect the origins with the parietal layer of protoplasm. Microsomes are seen to travel backwards and forwards along these threads from one origin to another, occasionally stopping and quivering half-way. The parietal layer is well-defined and thinner. It seems, too, as if part of the vacuoles get enclosed in the origin, which occasionally shows at either end a clear septum-like disk of refringent protoplasm, while the deeper part is

clearly vacuolated, richer in microsomes; and it is often possible in the living sporange to detect the chromatin body of the nucleus as a definite granule, lying in a clear space in the centre of the origin (Pl. XXVIII., fig. 19).

The above types of segregation grade into one another: thus I have figured (Pl. XXVIII., fig. 8), a "full" sporange of Saprolegnia, one part of which is segregated on the Leptomitus or zigzag type, another on the barrel type. So also in Aphanomyces we may find, in some parts of a sporange, a zigzag vacuole, between several successive origins, quite of the Leptomitus type; and such a case as this would make one hesitate as to the value of this distinction for the creation of a genus, were it the only one characterising Aphanomyces.

During this time the microsomes and oil-granules seem to leave the connecting plasma, leaving it hyaline, and concentrate thickly in the origins, thus making them very opaque; but in Aphanomyces granules continue to travel along the threads to the very end of the stage.

Stage of Swelling or Homogeneity.—The origins now contract, widening the interspaces and breaking most of the plasmatic threads, and at the same time become smooth on their free surfaces, which before were rough and granular. This stage lasts, at most, one or two minutes, to give place to Büsgen's 'homogeneous' stage, which Rothert calls the stage of 'swelling up' of the spores. The origins swell up, touch, and apparently fuse, the sporange becoming clear and brighter; the septum, previously concave, becomes convex, bulging into the sporange; and the rounded cap of the process becomes flat; the sporange has lost its turgescence (Pl. XXVIII., fig. 9).

Directly afterwards* vacuoles appear in the protoplasm: they come and go for some time (fig. 10). This is accompanied by a decrease in breadth as I showed, and in length as was demonstrated by Rothert.†

Cell-sap passes out and attracts bacteria, which perform a merry dance in the neighbourhood, as well as any Saprolegnian zoospores which happen to be about in the *second* stage of their activity, for in the first stage they are not attracted (this point was left uncertain by Rothert and Berthold). The cause of these phenomena is, as I first showed, the swelling up of the protoplasm, and its loss of resistance to osmosis; and I pointed out that

* *Loc. cit.*, "Formation and Liberation of Zoospores." † *Loc. cit.*, "Recent Researches," &c.

probably the Hautschicht and vacuolar wall break up at this stage, and become reconstituted later on. Rothert went further, and showed that this solution of continuity is itself due to the rupture of the protoplasmic investment of the cell-wall between the origins.

"This is easily seen in poor sporangia. In optical section the protoplasmic investment can be seen to rise and divide between two origins, and go half to either. At this moment the origins (now spores) become full of minute vacuoles, which diminish in number and enlarge as the spores swell, and the protoplasm becomes finely granular. It is here quite clear that the two processes, the vacuolation and the swelling up of the spores go hand in hand; the phenomenon of swelling is easy to understand, if we make the really obvious supposition that the protoplasm of the spore has a tendency to take up water. This could not hitherto have full play, so long as the 'Hautschicht' was continuous; but now it is interrupted at the points of severance, and, before its reconstitution, cell-sap is taken up into the protoplasm of the spores, and determines their swelling. The cell-sap so absorbed, or a part of it, is at once secreted in the form of the shifting vacuoles."*

But this account requires supplementing in one very important point. The question arises: does the parietal layer (*Wandbeleg*) *everywhere* rupture between the origins? Rothert seems to imply that it does so; but I think I must answer the question in the negative; for the swelling up of this stage starts at one part of the sporange, and extends gradually to the rest of the sporange; I have most frequently seen it begin towards the middle; and think it very probable that the rupture only takes place at one spot, the spore-origins then becoming even more closely connected on the whole, until their final separation.

The explanation which first suggested itself to me, before I knew through Rothert's Paper of the rupture, was the following:—

"The protoplasm is acted upon in two ways:—

"1. The tendency of the protoplasm (as a viscid body) to stick together into a single mass.

* It is obvious that the cell-sap, which is an "indifferent fluid," cannot alone determine vacuolation; to do this it must be diluted by the water from without.

"2. The tendency to aggregate around numerous centres to form the prominences ['origins' in our present nomenclature] *aided by the turgescence of the sporange*.

"If then the thinning at the intermediate bands went on to complete rupture at any one point, the turgescence would be lost; so the first force would overcome the second, now left unaided by the turgor lost for the moment, and would thus induce homogeneity, though the second force ultimately gains the upper hand in the next and last stage."

I went on to say that if this were the true explanation plasmolysis should induce homogeneity, and this not being the case I gave it up; but I failed to take into account the swelling up, which requires the absorption of water from without, and which consequently is absent in ordinary plasmolysis.

On the appearance of Rothert's Paper, while making reservations as to my priority on certain points, I fully accepted his views as to the complete separation of the spores, and his statement that the apparent fusion was due simply to their swelling; for he stated that in all cases he could see, or at least glimpse, persistent lines of separation. But the study of my balsam specimens has convinced me that my very first view, though incomplete, was correct as far as it went, and that the spore-origins, never completely separated in the preceding stage, do absolutely fuse together in "homogeneity." For the process of mounting in balsam involves a shrinking to about two-thirds the previous dimensions; and the shrinkage is perfectly regular when the mount is successful. Now if a film of liquid separated the origins at this stage, the effect of mounting would be to widen their interspaces and increase the plainness of their separation; and this is not the case: the protoplasm in the sporange is everywhere continuous (Pl. XXVIII., figs. 9, 10). Again, in the final separation of spores it often happens that we find large masses leave the sporange, which only after their exit break up into from two to four single zoospores. The only explanation of this fact is to be found in the account I am following here.

Let us examine the matter a little more closely. It is certain that touching surfaces of protoplasm inside a vacuole fuse where they meet, like the surfaces of any other viscid substances in a liquid that wets them. This is

easily seen in any object suitable for the study of protoplasmic streaming. It is only *external* surfaces of protoplasm, bounded by a Hautschicht that come in contact without fusion, and even to this rule there are many exceptions. Now, as we have seen, by the opening of the cell-sap cavity at a single point, turgescence is completely lost, with the result of the collapse of the viscid protoplasm and escape of the cell-sap, and it is impossible for water to enter and replace this with sufficient rapidity to convert at once the (problematical) "vacuolar wall" into a proper "Hautschicht." Hence I think my old view still holds good. When the rupture and collapse of the protoplasm occur, the origins are no longer held apart, and the thinned intervening bridges of plasma no longer distended, do now stick together into a sensibly homogenous mass. At the same time, the water which does enter and dilute the cell-sap to some extent, has a strong osmotic action on the protoplasm, no longer protected by a continuous Hautschicht, nor by contact with the cell-wall; it induces the solution of the microsomes, the fine vacuolation of the protoplasm, and its consequent swelling. At this stage indeed the texture of the protoplasm may be compared to that of the crumb of a loaf, with the nuclei diffused at intervals like currants through the spongy mass.

In Aphanomyces this stage proceeds somewhat differently. The first sign is the moving up of the origins towards one another, and their swelling up slightly so as to blend with the parietal layer of protoplasm, and to encroach on the vacuoles that separated them. At the same time the parietal layer quits the cell-wall in the zones of the vacuoles, so that, instead of forming a short tube between successive origins, it assumes the form of a dice-box, and this constriction increases till the successive origins are connected by only thin threads of protoplasm, in which granules, probably fatty, are still noticeable. The protoplasm of the origins shows a few large vacuoles, but I have not been able to satisfy myself as to their constancy of number. At no time is there any complete separation of the zoospores till at least the beginning of their exit from the tube. The origins do not swell up as much as in other genera; their microsomes persist, and that part of the connecting tube of parietal protoplasm which goes to each zoospore forms a sort of hyaloplasmic cap, of which the connecting-threads are prolongations (fig. D, 1, 2).

The final Fashioning of the Zoospores.—Grooves of demarcation run in from the periphery, and separate the spore origins anew, leaving at first bridges of junction; these, however, finally give way. Owing to the un-

Fig. D. 1.

Fig. D. 2.

Fig. *D* (Aphanomyces).—1. The zoospore origins united by shrinking tubes of parietal protoplasm.
2. The tubes forming hyaloplasmic caps and connecting-threads.

evenness of rate at which this work of demarcation goes on, the spore origins are at first partially isolated into connected groups of 2–7 (Plate XXVIII., fig. 14), and then these groups usually break up into what now may be called individual spores. As the vacuoles are nearer in refractivity to the protoplasm than the microsomes which have disappeared, the net result is a decided gain in transparency, which persists till the emptying of the sporange. The colour by transmitted light has lost its brownness owing to the fact that the rays which reach the eye following a less devious course are not so tinged by the protoplasm.

Nowhere has the study of well-fixed and stained specimens proved more advantageous than for the complete explanation of the shifting vacuoles, both with reference to their distribution and to their shifting character. The first effect of the homogeneous stage was to convert the protoplasm into a mere sponge; the vacuoles run into one another to form larger ones, and these discharge at the free surfaces, either towards the wall of the sporange or towards the now-growing lacunæ that separate the spores (fig. 14). At the moment of discharge the droplet so formed may indent two adjoining spores or lie at the angle where three meet (fig. 11). From a careful review of the specimens it may be fairly conjectured that the discharge of liquid always takes place by what are destined to be the contractile vacuoles of the mature zoospore, and by these only. The opening of these vacuoles at the surface is an additional proof of the thesis that the contractile vacuole does open in systole, and that its contents are not re-absorbed by the protoplasm. The following observation may be cited,

which I have published elsewhere*:—"Two sporanges of Saprolegnia opened at an early stage of the partial segregation of the protoplasm into origins. Part of the protoplasm in each slowly escaped and aggregated into rounded masses. The first discharged masses underwent the usual pathological changes and diffluence; the latter masses (from both sporangia) had already acquired the power of forming contractile vacuoles, that is possessed by zoospores; the numerous small vacuoles appeared and contracted regularly, lines of separation formed and deepened, and the masses divided into zoospores; these separated and swarmed, just like the protoplasm which remained in the sporange, though more slowly."

Owing to the increased transparency of the sporange as a whole, the planes of separation between the young zoospores are more easily glimpsed by low power objectives of good penetration, even when not absolutely in focus, than those between the origins in the earlier segregation; and so the observer has the impression that they are much more numerous, and, by parity, that each origin must give rise to more than one zoospore. But this would be inconsistent with the fact that the nuclei which are central in each origin, and which are present singly in the spores, remained unchanged through all these processes. Büsgen thought that he could detect indications of binary sub-division in the homogeneous stage in *Leptomitus lacteus* from the frequent approximation of nuclei in pairs, at this moment; and De Bary† put the matter even more strongly; but this is of course explicable by the fact that the young spores are frequently wedge-shaped and overlap. In the narrow sporange where two wedged-shaped spores overlie one another, possibly not even *yet separated after the homogeneous stage*, they will be seen with the two nuclei close together in a view from above. I have repeatedly counted the origins in slender sporanges before homogeneity, and

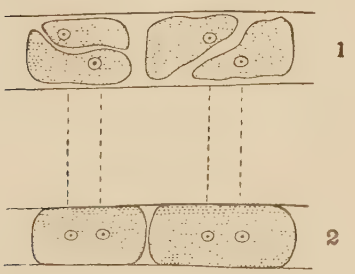

FIG. E.

Diagram of approximation of nuclei in pairs, simulating recent fission, in *Leptomitus lacteus*.
1. Side view, showing true relations.
2. Plan, showing false appearance.

* "Preliminary Note on the Contractile Vacuole," Brit. Assoc. Rep., 1888.

† "Morphology and Biology of Fungi," Engl. Trans., p. 74.

the spores on exit from the same, and found a close correspondence in their numbers; the slight divergences (at most 5 per cent.) being easily accounted for by the extreme difficulty in counting the origins with accuracy. The spore or spores which occupy the base of the discharging process are differentiated from it as follows:—

"One or more vacuoles appear at the base of the hyaloplasmic disk, and by their enlargement separate a terminal portion from the front spore, leaving one or two strings along which the hyaloplasm is retracted into the front spore. Next appear the cilia, as slow out-growths, at first short straight bristles with simple oscillations. The front spore has its cilia always at the front end next the process; but there appears no polarity about the others At the same time the spores manifest quivering (wackelnde) movements, increasing in strength till their discharge.

During this construction and development of the spores, they become warty, and some of the processes are abstricted (figs. 12, 12*a*). These lumps of protoplasm after independent movements are mostly absorbed (probably always) by the very spores from which they were separated; a few may be unabsorbed, pass out with the spores, and undergo diffluence,* but this makes no difference to the spores themselves. Rothert recalls similar processes described by De Bary in the formation of oospheres. This peculiar process is well shown in the figure. No explanation has been put forward of it hitherto; and it is with diffidence that I recall the kindred observations related by Berthold† with respect to the specialisation of the protoplasm between the zoospores of Cladophora, &c., as *periplasm*, which should by rights furnish these with a cell-wall, but which instead degenerates and disappears, or remains as fragments. In the Saprclegnieæ we may suppose that the separation of the zoospores is so accelerated as to anticipate the excretion of periplasm; the abstricted fragments would then represent periplasma, which, failing its proper purpose of forming membrane, is taken up again by the zoospore; and though the Saprolegnieæ appear so primitive a group as regards the Fungi at large, yet we may well believe them to have come from lower forms, like the Siphoneæ from the apocytial Cladophoreæ.

The conditions in Achlya are somewhat different from those of Saprolegnia

* This is not infrequent in Achlya.

† Protoplasma-Mechan., p. 302.

and Leptomitus, for the separation after the homogeneous stage is masked by the fact that the zoospores tend to approximate together within the sporange with their long axes parallel. This is due undoubtedly to a form of irritability which I have elsewhere termed "adelphotaxy," and which is fully discussed below in the section dealing with the discharge of the zoospores. I have already indicated its significance with respect to the non-persistance of separation during the homogeneous stage.

The cilia appear, according to Rothert, as slow outgrowths, at first short straight bristles with simple oscillations. The mature zoospore contains a "resting" nucleus, three pulsating vacuoles, and over one-quarter of its bulk is rich in what we have regarded as glycogen droplets distributed near the surface. In Aphanomyces the young zoospores, as they recover from their slight swelling, contract a little and become distinctly spindle-shaped, and are still united end to end by the protoplasmic thread that represents the remains of the parietal tubes between the successive origins.

SECTION 9.—The Discharge of the Zoospores.

In my Critical Abstract of Rothert's researches, as well as in my previous Paper, I have fully described the mode of discharge of the zoospores. In Saprolegnia and Leptomitus they swarm out, crowding towards the mouth of the process, and scattering as soon as they emerge; the last ones press forward with much less energy, often moving to and fro, and only quickening into a decisive rush for the exit when they approach it. In Achlya the spore-mass as it leaves the cell-walls frequently forms a cylinder, and presses out as a mass, not definitely separated into zoospores, till after exit; this cylindrical mass thins by this discharge towards the apex first, and so becomes conical; or it may become divided in the sporange, first breaking up transversely into sections, and these again break up into zoospores, which may remain united by thread-like processes while still in the sporange, or separate completely therein. On exit the spores of Achlya pass out as ovoid bodies, and remain at the mouth of the sporange in a hollow sphere which enlarges till discharge is completed: each spore as it passes out forcing its way between the others that have already taken position in the wall of the sphere. The zoospores after taking position rotate a

few seconds round their axes, and then very shortly round off and encyst. The fact that they resemble those of Saprolegnia in possessing a pair of flagella at one end was noted by Cornu, denied by Büsgen and De Bary, and, though confirmed by me, was expressly denied again by Rothert. I have never failed to see them at this stage by careful illumination or by staining with iodine; and Humphrey has also confirmed their existence in this genus.*

De Bary and Büsgen admitted, with some of their predecessors, that in all cases there existed in the sporange between the spores a substance which swelled up in water, and forced them out. In my first Paper I pointed out that the existence of this substance was based on no objective evidence; and that the facts were better explained by assuming the zoospores to be chemiotactic, and leaving the sporange under the stimulus of the more oxygenated water outside (aerotaxy). Rothert confirmed my disproof of the supposed objective evidence for the supposed expulsive substance; but as he had not seen the cilia in Achlya he could not admit the discharge to be due to the movements of the zoospores. He adduced certain experiments to show that they are not attracted to aerated water from boiled water. In my criticism I showed that his results were incomplete, and that even if they were adequate the hypothesis that the spores were repelled by the products of their own metabolism (pneumatotaxy) would meet the case. I may say now, supported by Humphrey's opinion, that I believe more strongly if possible that the exit of the zoospores is due to their irritability and chemiotaxy; whether it be positive aerotaxy or negative pneumatotaxy, I must leave undetermined for the present. Since I have used an iris diaphragm I have never failed to see the cilia in the discharging zoospores of the living Achlya, by narrowing the illuminating pencil. Moreover in the branched Y-shaped sporanges of Achlya with an exit on either limb (fig. B, p. 668) I have seen spores passing up one of the limbs stop short when a block occurred, go back to the junction, and then depart through the other limb; I have also seen that when a few of the front spores stopped short and encysted without leaving the tube, that the hinder

* Notes on Technique II. in "Bot. Gazette," vol. xvi., 1891; and "The Saprolegnieæ of the United States," p. 75.

ones passed on without disturbing these laggards. How such facts can be reconciled with the hypothesis of the expulsion of the spores by the swelling of a limpid mass, I leave to be explained by those who still maintain the existence of such a substance, which must have the same refractive index as water, must cease to swell on addition of alcohol, and not swell up on its replacement by water, and must yet show no difference of refraction all the time and which is affected by all stains in the same way as the ambient fluid; for such a substance must it be.

The peculiar conditions in Achlya may be explained by assuming the spores here to possess an additional mode of irritability, which I have termed 'adelphotaxy'; that is, they attract each other and tend to place their long axes parallel, provided the distances between them do not exceed their length. This may be readily seen if we blow or wash with a gentle stream the ball of zoospores as it forms at the mouth of the sporange, while spores are still passing out; in this way the zoospores are scattered to all distances from one another, and are seen to swim about for a short time, then to revolve on their axes, as they do in their positions in the hollow sphere they form when undisturbed at the mouth of sporange, and finally round off and encyst.

Professor G. F. Fitz Gerald, F.R.S., has suggested to me that this adelphotaxy is caused by superficial excretion from the surface of the zoospores, and consequent surface tensions attracting them by capillarity; this seems a valid physical explanation here. For in Achlya, and still more in the allied genus Aphanomyces, in which the zoospores also collect at the mouth of the sporange, the zoospores escape in a less differentiated state than in Saprolegnia and Leptomitus, the Hautschicht being practically non-existent in Aphanomyces. Under these conditions the protoplasm obviously will be freely acted upon by the ambient water, and the liquid immediately in contact with the escaping zoospores will be rich in dissolved substances, more abundant along the sides (if one calls them so in a cylindroid) than at the ends owing to the relatively greater surface exposed; and in any case elongated bodies approximating by capillarity would tend to place their long axes parallel.

Whether Professor Fitz Gerald's explanation is suitable to all cases

of adelphotaxy I cannot say. In Achlya the processes are essentially the same in the maturation of the zoospore as in Saprolegnia, and fragments of periplasm may often be seen left in the emptied sporange or carried out in the wake of the zoospores on their exit. It not infrequently happens, however, in this genus that even at the moment of exit the separation of the spores is incomplete,* and that some of them are really double, and only divide on exit or even on the resumption of activity after encystment. This imperfect separation of spores has been seen and figured by De Bary† in Aphanomyces, and by Leitgeb, in his Diplanes‡ (which is equivalent to *Saprolegnia ferax, monoica*).

But Aphanomyces presents features which, hitherto undescribed, clearly prove that the spores are discharged by a *vis a fronte*, since the phenomena to be described can only be explained on this supposition. I had indeed anticipated that, as in Achlya, I should here find cilia overlooked by De Bary, the sole student of the genus. But in my species flagella have escaped the detection of the highest powers and the most careful illumination, even after treatment with iodine or osmic acid followed by various aniline dyes. Aphanomyces is unique in having a sporange so narrow that the oblong spores lie compressed therein, and we might anticipate an exceptional mode of discharge. The hypha bursts at the pale apex; the foremost spore creeps out, and as it emerges its hinder end tapers more, and is drawn out; the filament joining it to the next spore lengthens a little; and then the next one follows, and so on. If owing to, say, a kink in the sporange a spore is arrested at one point, all the spores behind are immediately arrested, while the front of the arrested spore is drawn out into a long and irregular thread, such as we might draw out of very viscid black treacle full of bread crumbs: during this time the part of the spore behind the stricture shows no sign of compression; but the connecting thread between it and the one behind often slackens and becomes tortuous, as if it tended to thin out and elongate. We may readily trace the ordinary conduct of protoplasm forced freely through a narrow hole in the case where the opening of the sporange is unduly narrow in Achlya or Saprolegnia: there, on its exit, the free portion

* See "Pilzen," p. 82.

† In Pringsheim's "Jahrbücher," vol. ii., p. 176.

‡ *Ibid.*, vol. vii., p. 377.

of protoplasm at once expands in the water into a sphere, which enlarges as a greater and greater portion of the protoplasm passes through. Now it cannot be urged that the sporange-cavity contains a liquid differing in osmotic properties from water, for we know how instantaneously diffusion takes place through a cell-wall devoid of inner protoplasmic Hautschicht: all our micro-chemical methods prove this absolutely. Hence it can only be the pull exercised by the spore in front that draws out the thread connecting it with the arrested one in this peculiar way. Whether in other species of Aphanomyces the thread is sometimes absent, or whether it has only escaped notice, is uncertain. In the former case possibly the zoospores have flagella like Achlya; or they may creep out by the same amœboid action that clearly helps them on in the present species. Indeed I believe that the escape of the front zoospore is here, as in all cases where the exit hole is narrow for the time being, due to an amœboid crawl through the constricted orifice of the sporange.

SECTION 10.—**Ultimate Fate of the Zoospores.**

Encystment.—The zoospores always encyst either—1, within the sporange; 2, at the mouth of the sporange; or 3, in the medium after a short swarming. The *first* condition is normal in Dictyuchus, and in Thraustotheca (where the sporangial wall deliquesces at once, leaving a mass of spores behind. It occurs frequently towards the centre of thick old tufts and in ill-aerated cultures swarming with Flagellata, Ciliata, and Schizomycetes; and this occurrence is one of the grounds on which I have founded the hypothesis of chemiotaxy as the cause of discharge in these genera. Sometimes, while the majority of the zoospores escape, a few laggards remain and encyst within the sporange. A sporange, in which all or the majority of the spores remain and encyst, is termed, from the honeycomb formed by the cyst-walls, a 'dictyosporange.'*

The *second* condition is normal as described above in Achlya and Aphanomyces; while the *third* prevails in Saprolegnia and Leptomitus.

* Since the majority of the zoospores of Saprolegnia and Leptomitus lie, after their first encystment, at the edge of the hanging drop, or in contact with air-bubbles, I think they must possess, in this stage, a certain reaction to surface tensions or "thigmotaxy."

Diplanetism.—In all three cases the zoospores, after a time, usually escape from the cyst through a small hole; though in the dictyosporange they may germinate directly, especially if the cyst-wall is separated by an interval from that of the sporange (Pl. XXVIII. fig. 16): on their escape they differ much from their pristine condition and behaviour. They resemble the zoospores of the Peronosporeæ in being kidney-shaped, with a single nearly central contractile vacuole, and two long flagella projecting from the notch, an anterior 'tractellum,' and a posterior trailer or 'pulsellum.'

In this conditio . they swim for a prolonged period, and are, as was shown by B. Stange,* strongly chemiotactic, being attracted by solutions of meat-extract, lecithin, phosphates of alkalies and alkaline earths, and free phosphoric acid; acetic and tartaric acids were but slightly attractive; other acids and free alkalies, nitrates, and various other salts, grape sugar and milk sugar, glycerine and leucine, were indifferent. They show positive aerotaxy to oxygen. This will explain the fact that they are frequently found after the second encystment that always ensues sticking to the bodies of dead insects, &c.

Pringsheim denies that the zoospores of Leptomitus are diplanetic; but Humphrey has adduced evidence to the contrary.

I gave in my first Paper the following suggestion as to the physiological value and filiation of diplanetism:—"The zoospore, on leaving the sporange, has enough reserve material to carry it a certain distance, and to enable it to germinate. In the first swarming [in Saprolegnia] the zoospores get scattered; and then during the long stage of encystment, the further work of dissemination is effected by the movements of the water at no cost to the organism. And we must bear in mind that a considerable amount of dissemination is needed, lest the zoospores should be uselessly attracted to the host on which their parent thallus is living.

"In Achlya [and Aphanomyces] the limited first swarming forms a sort of globular colony of resting spores, easily broken away as a whole by a slight impact, as of a dead animal floating down stream. The swarmers when liberated form a host of invaders, effecting rapid and complete infection, which isolated ones might fail to do.

* "Ueber Chemotactische Reizbewegungen," in Botan. Zeitung, vol. xlviii. (1890).

"In Dictyuchus [whose sporanges, filled with resting spores, break away also on slight impact or agitation] the resting state of Achlya is reached without the expenditure of energy required to swim to the outlet of the sporange; a further economy is thus effected."

Germination.—In this process a vacuolation of the cytoplasm is a marked concurrent of the emission of the germinal hypha. The nuclei frequently, but not always, divide early; in one case I have figured (Pl. XXVIII., fig. 16) three nuclei in a spore which had not yet emitted a germinal hypha like its sisters. Often two hyphæ are given off when one nucleus passes to either (Pl. XXVIII., fig. 17). These hyphæ, like the vegetative hyphæ, are strongly chemiotropic, bending towards any substratum (such as a fly-leg) fitted for their growth as soon as they come within a certain distance therefrom. This shows beautifully in infected fly-legs, where one joint has abundant germinations and the other not; up to a certain distance from the infected joint the hyphæ are at right angles to it, and then they show a sudden kink, so as to direct the apex straight towards the other joint. Similar chemiotropy is of course everywhere shown by the haustorial hyphæ emitted from the base of the ordinary extramatrical branches.*

Since the above was written, Myoshi, in his Paper on "Über Chemotropismus der Pilze,"† has stated definitely that the hyphæ of germinating zoospores respond to the stimuli of various chemical substances in solution in weak gelatine or water. The tubes were attracted by phosphates, ammonium nitrate tartrate and malate, cane and milk sugar, maltose, dextrose, and levulose, dextrin, asparagin, urea, lecithin, meat extract, peptone, and decoction of plums. They were repelled by phosphoric acid, calcium and potassium nitrates, sodium and potassium chlorides, ammonium carbonate, sodium malate and butyrate, and alcohol (0·5 per cent.). Glycerine and ammonium chloride were indifferent.

* Stange (*op. cit.*, p. 140), who experimented according to Pfeffer's method with capillary tubes containing meat-extract, under these conditions found the growth more vigorous as the hyphæ came into the diffusion area, but never found a divergence in direction towards the tube-mouth. But the observation of ordinary cultures is quite sufficient to show that chemiotropy does exist here.

† In Botan. Zeitung, I. Abth., Heft I., 1894.

Section 11.—The Sexual Reproduction.

Earliest Literature.—The full study of the sexual organs must necessarily precede that of the recent literature on the subject which is almost entirely controversial. We may note that the sexual organs were early recognised as a distinct mode of reproduction; and Pringsheim, in his classical Memoir on the Sexuality of Algæ,* was the first to recognise their true character. He, however, suggested that motile spermatozoa were emitted from the antheridia.† Max. Cornu, in 1872, demonstrated in his classical "Monographie des Saprolegniées‡" that the antheridia emitted "fertilizing tubes," which reach the oospheres; and in Pythium, which he considered a member of this group, he described and figured the passage of plasma from the tube into the oosphere, fertilizing it like the pollen-tube fertilizes the ovum of flowering plants. From this he inferred that the same took place in the true Saprolegnieæ. He also showed that Pringsheim's "antherozoa" were really Chytridian parasites. Nine years later appeared De Bary's work, which gave a complete description of the chief phenomena visible under moderate powers in the living specimen, and opened up a new period of controversy and research.

The Sexual Organs.—These consist of oangia and antheridia. The former are enlargements of the hyphæ, which may be terminal, or on lateral branches, or intercalar. In the last case they are cylindrical, frequently with a cask-shaped swelling; otherwise they approach the spherical form. In these oangia protoplasm accumulates, until the communication with the bearing hypha is closed by the formation of a septum in the same way as in the zoosporange. The contents of the oangium separate into *oospheres* which ripen into *oospores*, which are finally spherical bodies with a thick cell-wall and a single "resting" nucleus. The antheridia are swellings on thin hyphæ which grow on to the wall of the oangium, and become separated from the hypha by a septum. Each antheridium (and there may be many to

* "Ueber die Befruchtung der Algen," in Monatsber. d. Akad. d. Wissenschaft. zu Berlin, 1857; translated in Ann. d. Sc. Nat. Bot., sér. 4, vol. iii.

† "Die Saprolegnieen," in Jahrb. f. Wiss. Bot., vol. i. (1857), and "Nachträge zur Morphologie d. Saprolegnieen," *ibid.*, vol. ii., 1860.

‡ Ann. des Sc. Naturelles, Bot., sér. 5, vol. xii.

each oangium*) has the power of emitting a "fertilising tube," which penetrates the wall of the oangium after its contents have been resolved into oospheres. These tubes touch the oospheres; but in all the species examined by De Bary (with a doubtful exception), Marshall Ward, and myself, they remain closed and fail to exercise any influence on them ; no difference is seen in the character or germination of the oospheres, whether antheridia and "fertilizing tubes" be formed or not. Pringsheim's account, which differs widely from the others, will be examined below.

The Formation of the Oangia.—This process is nearly the same as that of the zoosporangia: protoplasm, rich in fat granules, accumulates in a given part of a hypha—either the end of a long one, or more frequently the apex of a special lateral branch; and sometimes, especially in old cultures, intercalar oangia are formed. In some cases the arrangement of these is characteristic of the species, especially in the very interesting species *Achlya racemosa* (which I have only once seen), where they are lateral, racemose, acropetal; on *A. recurva* they are also lateral and racemose, but the succession is less regularly acropetal, and the stalk of each oangium is recurved; in the other species it is difficult to observe more than a generally racemose arrangement at first, which, as fructification advances, gives place to every irregularity imaginable.

The oangium is in some species spherical from the outset, but in the Achlyas it is frequently fusiform when young, though increasing size and turgescence usually bring about the disappearance of the taper apex. The septum is rarely at the very base of the sphere; it usually lies lower down on the stalk, so that the oangium has a short cylindrical base. The protoplasm usually fills the oangium completely: De Bary says *always*, but in some cases there is a central vacuole from the first. It is remarkable for the abundance of fat granules, and also for the numerous spherical "resting" nuclei which it contains. After a time numerous clear spots appear in the dark protoplasm, which gradually pass

* The term *oogonium* is used to denote a cell which by true and repeated division produces a brood of oospores. The term *oangium* (by parity with sporangium) is better suited to the present case, where an apocytium resolves itself into one or more oospheres by a process very distinct from cell division.

to the centre and open into the central vacuole, which enlarges in this way until the protoplasm is reduced to a thick peripheral layer. As these vacuoles have often the size of nuclei, and are inconspicuous in balsam specimens, I erroneously interpreted them as being nuclei in my note in the Comptes-rendus,* an error which I corrected in the margin when distributing the "tirages à part," immediately after publication. The nuclei lie evenly distributed between the vacuoles at first (Pl. XXIX., fig. 33); but, as the intervening protoplasm is gradually reduced, they are brought closer together, often in files or lines (Pl. XXIX., figs. 29, 30, 36, 37, 38). The chromatin of the nucleus is reduced and its hyaloplasm becomes very pale, while the nuclear wall, though seen in crushed specimens, is now undistinguishable in colour from the surrounding protoplasm of the uninjured oangium. Hence the study of the nuclei and their behaviour at this period becomes enormously difficult; and it is only possible under the best possible conditions of preservation staining and microscopic apparatus. As the narrowing of the protoplasm goes on, the number of the nuclei is reduced; their size is no longer uniform; they are often seen approximated in pairs of even or uneven size; they contain more than one chromatin granule—from 4 to 20, the number being usually greater as the stage is more advanced. The only interpretation I can place on these facts is that the nuclei fuse when they approximate; and that the nuclein masses, though reduced in size, long retain their individuality in the compound nuclei so formed.†

It might be conjectured that the multiple nuclein granules point to karyokinesis, and so they do in the etymological sense of 'nuclear disturbance,' but the reduction in number of the nuclei is a proof that the 'nuclear disturbance' is here one of fusion, not of multiplication. Whether any of the nuclei disappear and dissolve into the protoplasm, as sometimes stated, it is, of course, impossible to tell; but I do not think so, though the idea had repeatedly crossed my own mind. The great number of nuclein masses at this stage is quite sufficient to explain the reduced number of nuclei. The size of the individual nuclein masses when they occur in numbers at these

* Recherches s. l. Struct. des Saprolegniées, 1889.

† In the latter case the real number must be much higher than what we can count, owing to superposition.

late stages is enormously reduced, as shown by the figures. It is interesting to compare matters in Pythium and Peronospora, where, according to Wager, a great multiplication of nuclei by mitosis takes place in the oogonium. The differences are so great and so obvious that we cannot ascribe them to probable errors of observation on either part, but to the radical difference between the types of oangium and to the different formation and fate of the oospheres in either case. In Peronospora, however, the nucleus of the oosphere or "female pronucleus" appears to be formed by the fusion of three (all the others going to the periplasm); and so far this is a meeting point between the processes in the two groups.

The Formation of the Oospheres.—This takes place in three stages, well described in De Bary's classical Memoir; the formation or segregation (Ballung) of the origins; their separation (Trennung), and the rounding off into oospores (Glattung). In the first stage the protoplasm, which hitherto formed a uniform layer, now becomes uneven, rising towards the centre into as many elevations as there are destined to be oospheres; and these are united by a continuous parietal layer closely applied to the oangial wall. The process resembles that of the formation of the zoospore origins: the whole of the protoplasm is in slow, but constant motion: the origins are frequently united by strands which stretch across the large central vacuole, and along which bright granules glide to and fro. In Saprolegnia each origin has a central nucleus as a rule; this has the character of a "resting-nucleus," and is, I consider, formed by the union of the nuclei with numerous chromatin granules which have now united into a single nuclein mass. This nucleus was termed Kernflecke by De Bary, who saw it as a clear spot differing in refractivity from the vacuoles, and surmised its true nature. In Achlya, however, the process of nuclear union is more delayed: several nuclei occur in each origin, and are scattered irregularly through it, with no definite position (Pl. XXIX., figs. 30, 34). It is difficult to believe that the Ballung can take place without some nucleus near its middle to act as a centre for the forces; but I must admit that I have failed to find any.

After a time, as Rothert and Berthold first described in detail, the separation takes place, the parietal layer thinning off, and giving way at one spot first; the rest of the layer separates from the wall owing to loss of

turgescence, and the origins swell up a little, becoming alveolate or spongy with innumerable vacuoles; but this does not go on to the homogeneity of the zoosporange; and immediately afterwards the separation becomes general, and the "origins" have become "oospheres."

The oospheres now show the same wavy amœboid movements at the surface as we saw in the young oospores. Some of the pseudopodia separate completely; of these the majority are soon taken up again, but some may remain, of no further import, in the oangium, and these, I believe, represent in part the "Spermamoeben" of Pringsheim. The surface of the oosphere soon becomes smooth, with a distinct clear Hautschicht (more or less visible before), and the contents are dark with oil granules, and contain a few large glycogen drops.* Immediately under the Hautschicht numerous vacuoles appear and disappear: by means of these the greater part of the water taken up at the stage of separation is got rid of; and in this way the oosphere diminishes in size. At this period, or, to be precise, immediately after the separation of the protoplasm from the wall of the oangium, the antheridium sends its tube down into the cavity; this tube may dent an oosphere, but does open. Immediately after a cellulose wall develops on the oosphere, and becomes multilaminar as it thickens. The fat granules in the oospore now approximate and fuse into larger drops and then into a central drop, which is however, sometimes traversed by plasmatic threads, and the nucleus is pushed to one side. De Bary observed that the fat globule appears less refractive than is usually the case, and suggested that the fat might be mixed with other materials. But he overlooked the fact that fat drops are rarely seen embedded in so dense a plasma as here; and this is quite enough to account for its somewhat dull appearance. The glycogen drops break up into smaller ones scattered through the peripheral plasma. In some species of Achlya, the fat is more irregularly dispersed though the plasma, frequently forming a lateral drop, and the nucleus is then central.†

* As first described by Dangeard ("Recherches Histologiques").

† Dangeard, who failed to follow the nucleus from origin to mature spore, has suggested that an oil-drop stained by pigment may have misled me. This was clearly impossible, since my methods involve the extraction of all oil; and I am the more surprised at this suggestion, as, at the time, my methods had been published at the Paris Botanical Congress of 1889 (see "Bull. Soc. Bot.," France, vol. xxxvi.).

As above mentioned, in Saprolegnia the origin of the oospore is uninuclear in the stage of Ballung (Pl. XXIX. fig. 26), and uninuclear it remains to the maturity of the oospore. In a few cases, however, I have seen two nuclei of uneven size in the young oospore; and from this I might have inferred a sexual fertilisation, had not the oangium in these very cases happened to be free from all signs of antheridial branches, let alone 'fertilising tubes' (Pl. XXIX. fig. 25). In Achlya it frequently happens that the oosphere retains two nuclei till a very late period, long after a thick cell-wall is formed; and in this case the nucleoplasm suspending the chromatin mass is sometimes curiously radiate in optic section (Pl. XXVIII. figs. 23–4. The radiate appearance is not shown here, but is identical with that of the left-hand nucleus in Pl. XXIX. fig. 44). Sometimes in either genus, presumably owing to imperfect separation during Ballung, an oospore occurs of exceptional size with two distant nuclei, and sometimes two oil-drops. Such 'giant spores' are paralleled by similar formations in the zoosporange, as we have noted above.

The Antheridia.—The 'nebenäste' or male branches vary in position with the species; sometimes they spring from the stalk of the oangium or from the large hyphæ which form the peduncle of the oangial raceme; in other cases they spring from distinct hyphæ, even probably from distinct plants, though this is difficult to ascertain. They are long, tortuous, and usually not very full of protoplasm. When the antheridial branch reaches an oangium it forms an enlargement resting closely on it, which becomes separated off by a basal septum, and forms the antheridium. The number of antheridia on a single oangium varies; sometimes in Achlya they form a complete investment. The protoplasm contained is clear, not very rich in fat, but containing protein granules as well as nuclei: these last multiply by division, as shown by the small size and large number of those found in the 'fertilising tubes.'* The antheridia contract a very close union to the cell-wall of the oangium; and it might be conjectured, as an alternative hypothesis, that fertilisation takes place at this stage; but the cell-wall remains unchanged, and the shrinkages that take place in preparation show clearly that nothing but osmotic interchange of liquid

* This is a sufficient answer to the doubt that Humphrey has thrown on their division.

can take place, and that no formed element passes from the one organ to the other (Plate XXIX., fig. 28).

That no outgrowth from the antheridium takes place at this stage may be partially explained by the turgescence of the oangium. It seems pretty clear that, as De Bary conjectured, some chemical stimulus of liquid diffusing through the oogonium determines the growth of the male branches towards the oangia, and the formation of antheridia thereon; but this is insufficient to determine an ingrowth against the strong osmotic pressure of the parietal layer against the oangium; and possibly the secretion that determined the stimulus under which the antheridial branches reach the oangium ceases when they touch it. When the rounding off of the oospheres is completed, a new stimulus arises, and the resistance hitherto produced by turgescence ceases; the antheridium (or antheridia) sends a tube through the wall of the oangia, which, as above mentioned grows down to an oosphere and lies close against it. My own observations on the living specimen are imperfect; but De Bary has seen, in some cases, the oospheres dented by the tube, and at the point of contact the former often shows a lenticular spot from which the granules have receded, recalling the receptive spots of oospheres whch are destined to be fertilised. But the male tube always remains closed and turgescent: when it shrinks under the action of *dilute* ClZnIK, and in my preparations in oils or balsam, it is seen to be closed everywhere, and to have formed no organic union with the oosphere. In a short time the tube swells at the point of contact, and then grows out laterally into a tube, which continues until it meets with another oosphere, or winds irregularly through the oangium, sometimes piercing the wall again to emerge on the outside. This is a complete proof that in none of the species of Achlya and Saprolegnia examined by De Bary* and myself does any fertilisation occur at this stage. And as the oosphere (*oospore* as we may now call it) immediately after acquires a cell-wall which thickens rapidly it is certain that the last chance of fertilisation is now past. Our species are undoubtedly 'apogamous': the presence of antheridia and fertilising tubes brings about no fertilisation, and their absence does not affect the normal formation of the

* With the *possible* exception of his *S. caudata*. See "Bot. Zeit.," 1883, p. 43.

oospores: the male organs have lost their function, and their absence is a matter of indifference to the plant.

The *à priori* objection may be started, that it is inconceivable that so much good nourishment should be wasted as goes to these male organs. But the immense superabundance of masculinity in nature is notorious. How many millions of coniferous pollen-grains are wasted, for the few that serve a useful purpose? If a species of Pinus became apogamous, what is there to prevent the continued propagation of male trees, since the gain to the species by their suppression would be infinitesimally small. How many spermatozoa go astray for one that fertilises an ovum, in the majority of Vertebrata? The *à priori* objection then has nothing in it.

The above account of oospore-formation is essentially De Bary's, supplemented and confirmed by the cytological knowledge which modern technique has put in our reach. My observations thus confirm De Bary's view, and supplement it by the cytological details, which we shall see in the sequel afford a satisfactory explanation of the needlessness of the antheridia for fertilisation. But we must turn aside to consider the recent discussions on the subject, dating from De Bary's great Memoir in 1881, which emphatically denied the transference of any substance at any stage from the antheridium or its tubes to the oangium or the oospore. This was confirmed by Marshall Ward in 1883.*

Consideration of Pringsheim's latest Observations and Interpretations.—Meanwhile Pringsheim, who was one of the first discoverers of the antheridia, and to whom we owe a great debt for the indefatigable zeal and perseverance with which he has studied this group and so many others, published in 1882 a research† which re-opened the question. Pringsheim, working partly on fresh material, partly on material fixed by ZnClIK and stained with aniline blue or carmine, made out the following state of things. At a late stage, when the oospores have already a thick double wall, he saw and figured an attachment of the fertilising tubes of the antheridium to a papillar outgrowth of the spore. He also noted in

* "Observations on Saprolegnieæ" in Q. J. M. S., July, 1883.

† "Neue Untersuchung über den Befruchtungsakt der Gattungen Achlya und Saprolegnia," in Sitzungsberichte der Akademie der Wissenschaften zu Berlin, 1882, vol. ii., p. 855.

the antheridium more or less individualised portions of protoplasm, which show amœboid movements, and stain deeply; these, he says, cannot be identical with the nuclei of Schmitz*, as they are spheroidal, not lenticular. He further found that these, or similar bodies, escaped like small amœbæ through the gelatinous walls of the tumid free ends of tubes emitted from the antheridium, whether the fertilising tubes themselves within the oangium, or similar tubes emitted from the back of the antheridium into the surrounding water. He infers from these facts that these bodies represent spermatozoa, and terms them spermamœbæ. Zopf pointed† out that the individualised bodies escaping from the swollen ends of tubes were most probably amœba states of a Monadine parasite, and in this matter there can be no doubt he is correct. The external tubes, on the whole, are exceptional; and the peculiar dilated ends figured are undoubtedly pathological. It is impossible to conceive a normal antheridium sending out its fertilising tubes at random, and discharging them into the water or fusing and uniting with the oosphere indifferently. Again some of Pringsheim's spermamœbæ—those he figures in normal healthy antheridia (Pringsheim's figs. 4, 6, 8)—are certainly nuclei, which, as I have shown, are here spherical; and his figs. 13, 14 show probably normal conditions of plasma and nucleus.

As regards the union of the fertilising tube with the oosphere, I must express my want of faith in the figures as true interpretations of the microscopic pictures by the artist (Carl Müller) and the author, and attribute the apparent union to imperfect focussing. For the specimens figured have been stained with aniline blue; and while the plasma of the antheridium and fertilising tube is represented by a deep blue with darker nuclei, that of the oospore still retains the brown of the iodine. This is decisive proof that there is no protoplasmic continuity between the two, such as is figured; for in that case the aniline blue would have been carried into the oospore.

* Who was the first to demonstrate the nuclei in this, as in so many groups of Thallophytes.

† "Ueb. Parasiten in den Antheridien, Oogonien und Oosporen der Saprolegnieen," in Bot. Centralblatt, xii. 356; and "Erwiderung," *ibid.*, vol. xv., 156. See also Pringsheim's two inconsistent answers—"Ueber die vermeintlichen Amœben in den Schläuchen und Oogonien der Saprolegnieen," in Bot. Centralblatt, vol. xiv., p. 378; and "Nachträgliche Bemerkungen z. d. Befruchtungact von Achlya," in his own Jahrbücher, vol. xiv., p. 111.

The wall of the oospore is, at the stage figured, singularly impervious to stains, as I know too well; but the smallest breach of continuity would allow their free action. And the fact that author and artist have overlooked this anomalous colouring, which could not escape the attention of anyone accustomed to work with high powers on stained specimens, leads me at least to doubt the objective reality of the papilla figured on the oospore, and ascribe it to the false interpretation of the optical effect due to the passage of the fertilising tube *over* the margin of the spore. In the absence of any statement as to the mounting medium, we may conclude that it was probably water or glycerine, in which such deceptive appearances would be most likely to occur. He figures the existence of two nuclei in a (young thin-walled) oospore of Achlya as a proof of fertilisation; but we know this is not uncommon in cases where there is no antheridium formed; and I have figured one such case even in Saprolegnia (Plate XXIX., fig. 25), where it is less common.

In Pringsheim's fig. 12 *a* he represents two advanced thick-walled oospores in a state of fatty degeneration, which he ascribes to the fact that the fertilising tube, as shown, does not reach them; but Zopf ascribes this to parasitic infection, and my own observations sustain that view; for in an oangium with ripe perfectly formed oospores, I have seen on the living culture one after another fall victim to the disease, which cannot therefore be due to non-fertilisation.

I have now examined Pringsheim's case step by step, and shown the invalidity of his facts and figures to prove his case.*

The Correlation of the Oospore Formation in Saprolegnieæ with other Fertilisation Processes.—The apogamy of Saprolegnieæ does not however imply a complete absence of processes corresponding with fertilisation. The essence of ordinary fertilisation consists in the union of two cells, cytoplasm to cytoplasm, nucleus to nucleus (and in most cases, archoplasm to

* De Bary, in a controversial Paper ("Zu Pringsheim's neue Beobachtungen," &c., in Bot. Zeit., 1883, p. 38) rejecting most of Pringsheim's views, admitted the bare possibility of fertilisation in *Achlya polyandra* and *Saprolegnia caudata*; but this admission was based on the evidence of Pringsheim's figures, which are, as we have seen, inadequate. He rejects *in toto* the sperm-amœba hypothesis.

archoplasm). In this way is formed a new complex cytoplasm, nucleus, etc., which, as such, have never been associated before. In Saprolegniæ the nucleus of the oospore is formed by the fusion of many nuclei, which possibly have wandered from different parts of the plant, and which are, in any case, essentially different from all those formed by repeated nuclear fission in the previous life-history of the plant.

In the allied order Peronosporeæ we see an approach to this condition; for the nucleus of the oosphere is, in many cases at least, a fusion-nucleus formed by the union of several. If this process were carried a stage further, it is easy to see that the additional effect of a male nucleus (derived as it so frequently is from part of the same hypha as the oangium) would be of little or no value; while yet the stimulus of the neighbourhood of young female organs would still determine the continued formation of antheridia and antheridial tubes.

Obviously such endogamous nuclear unions cannot occur in plants with distinct walls partitioning them into uninucleate cells. But we do find parallel cases in other apocytial plants. In the Dasycladeæ the nuclei fuse several together in a gametangium, and round the single fusion nuclei the protoplasm aggregates to form uninuclear gametes, which pair with those of other plants. In Derbesia, zoospores with fusion nuclei formed in precisely the same way have apparently lost the faculty of pairing, and develop directly.* If Dasycladus were anisogamous, with smaller male gametes, the relations of these two genera of Siphoneæ would be absolutely comparable with those of Peronosporeæ and Saprolegnieæ.

In Uredineæ† the teleutospore is bicellular; in each cell the nucleus divides; and then in the cell the two sister nuclei fuse again, so that each of the two resting spores of which the teleutospore is formed has a fusion nucleus. And in Ustilagineæ each spore is primitively binucleate; but

* See Wille, in Engler and Prantl's "Pflanzenfamilien," I. Teil, Abtheilung 2, pp. 129, 152. The original observations are, I believe, in both genera due to G. Berthold.

† Dangeard and Sapin Trouffy, in Comptes-rendus, vol. cxvi., 1893. Felix Rosen also figured the fission of the nucleus of either cell into two, and conjectured their ultimate fusion—"Die Kerne und die Membranbildung bei Myxomyceten und Pilzen," in Cohn's Beiträge, vol. vi., pp. 257–8.

becomes uninucleate by fusion of the nuclei.* In Basidiomycetes the large nucleus of the basidium (which gives rise in the sequel to the several nuclei of the basidiospores) is formed probably by the fusion of numerous minute hyphal nuclei, first into two, and then into one.†

Since in neither of the above groups of Fungi has any other karyogamic process been made out, we can see that the paragamous condition of the Saprolegnieæ, where endokaryogamy has replaced external fertilisation, is no isolated phenomenon. If this condition appears strange at first sight, it is because complete research on these points was impossible until the advance in technical microscopy had given us the means of cultivating a hitherto untilled field.

In most animals the ovarian egg divides at maturity into a brood of four, three of which are functionless and are termed polar bodies, while the fourth is the functional oosphere which is usually fertilised by fusion with the male cell the spermatozoon. In many cases of so-called parthenogenetic ova, the second cell-divisions remain incomplete: the cell nucleus divides as if to give rise to the nuclei of a polar body and an oosphere, respectively; but then the former nucleus moves back and fuses with the latter, playing the part of a male to its sister, just as we have seen in the Uredineæ.

The consideration of these 'paragamic processes' has led us to reject altogether the views that have been put forward to show that the function of fertilisation is the mixture of *different* ancestral elements or tendencies. The sole *universal* interpretation of these processes is that the formation of a 'fusion nucleus' is of definite advantage to the organism at such crises in its life-history as are marked by the "sexual" reproductive act. This much is definitely proved, though the explanation of the possible mode of

* Dangeard, P. A., in Comptes-rendus, 1893.

† Rosen, *op. cit.*, p. 259. Wager, who has given a fuller account, finds in the young basidium of *Amanita stercorarius* 2 nuclei, in *A. muscaria* 2–3, larger than the hyphal nuclei, and possibly due already to the fusion of several of these. The 2–3 nuclei fuse into a single basidial nucleus, and by its two successive divisions the nuclei for the 4 basidiospores are formed ("On Nuclear Division in the Hymenomycetes," in Annals of Botany, vol. vii., 1893). While correcting the proofs of this Paper I learn that Dangeard finds that the primitive nucleus of the ascus in Ascomycetes is also a fusion nucleus.

benefit which I have put forward is merely a provisional suggestion;* but during the four years that have elapsed since it occurred to me, I have seen no reason to doubt its applicability.

It is interesting to note that the nucleus of the oosphere must be formed of many more chromosomes than is the norm of the group, and this tells very strongly against the assumption that their number has anything essential to do with fertilisation. Probably, as Dr. Strasburger suggests in a letter, a reversion to the ancestral type takes place in the germination of the oospore, and the original number of four chromosomes reappears on the first nuclear division. This opens out a new and difficult field for research; and I wish I had been able to break ground therein. But anxiety for completeness, combined with the perversity of one's cultures, which show the phenomena or the species one wants (as the case may be) just when it is impossible to work at them, have already delayed this publication far too long; and when it was nearly ready eighteen months ago, I lost some of the most important figures, and had to work them up afresh. I must therefore reserve this research for a later date, unless some other cytologist anticipates me.

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EXPLANATION OF THE FIGURES.

[All the figures are taken from views with Zeiss's apochromatic oil immersion objective, 2 mm. (1·3 N.A.) and compensation oculars. The figures on Plate XXVIII. (except 19–23) are copies of an enlargement from a photographic lantern-slide of a beautiful water-colour drawing by my friend and former student, Dr. J. C. MACPHERSON, of Wadsley Bridge Asylum, the original having been lost, and have been retouched by comparison with the first sketches and the preparations themselves; all these preparations are mounted in balsam, and the oculars used were 8 and 12 (with the exception of figs. 18, 20, 23, 24, which were drawn under ocular 18). The other figures are mostly from mounts in essential oils, and have been prepared from my own camera drawings.]

PLATE XXVIII.

Figure

1. Hypha of Saprolegnia injured by the action of water; the microsomes have disappeared, and the cytoplasm has a "foam-structure"; the nuclei have aggregated in groups. (The alveolar external layer has not been correctly interpreted on the plate by the lithographer.)

2. Young sporangial hypha of Saprolegnia, which has ceased to elongate; cytoplasm richly vacuolated, and still scanty in proportion to the nuclei.

3, 4, 6. Hyphæ of Saprolegnia still elongating, and showing stream-lines with nuclei.

5, 7, 8, 15. Young sporanges of Saprolegnia, showing various stages of the segregation of the spore origins; the cytoplasm has much increased in abundance. Fig. 7 shows the retreat of the protoplasm from the discharging process. Fig. 8 is a 'full' sporange, with a combination of the Aphanomyces and the Leptomitus types of segregation.

9–12, 14. Stages of the formation and isolation of the zoospores of Saprolegnia. Fig. 9, "homogeneous" stage, with very numerous and minute *rounded* vacuoles (figured too large). Fig. 10, stage of "shifting vacuoles." Fig. 14, imperfect separation into 'blocks.' Fig. 11, separation of zoospores complete; excess of liquid being discharged by contractile vacuoles. Fig. 12, stage of rounding off; the zoospores are more or less warty and amœboid, and some of the cytoplasmic pseudopodia are seen to be abstracted. Fig. 12*a*, one of the same zoospores under a higher power.

Figure

16. Dictyosporange of Saprolegnia. The zoospores, having failed to escape, have encysted *in situ*, and are now germinating; cytoplasm much vacuolated.

17. Two free zoospores of Saprolegnia germinating after encystment; cytoplasm much vacuolated.

18. Nuclei of large hyphæ of Achlya; the three uppermost are in the later stages of kinesis.

19. Zoosporange of Aphanomyces showing segregation; taken from the *living specimen* (Oc. 8).

20. Nuclei of vegetative hyphæ of Saprolegnia. At the right hand are seen two young sister nuclei, in which the four chromosomes are not yet aggregated into a single clump (Oc. 12).

21, 22. Two oospores of Achlya; the nucleus is still central, and the oil (dissolved out) traversed by numerous plasmatic threads. Two nuclei, not yet fused, are seen in fig. 22 (Oc. 12).

PLATE XXIX.

23, 24. Nuclei of Achlya in various "active" states. The three lowest right-hand figures show stages of kinesis—the middle one showing an equatorial plate in which, by focussing, I counted eight chromosomes; the nucleus above shows two groups of four each (metakinesis), and was seen obliquely in the specimen; the under nucleus seen end on shows four (double ?) granules (Oc. 12.)

25. Young oospore of Saprolegnia, which has, exceptionally, two unequal nuclei; no antheridial tube had reached the oangium in which this was contained.

26. Young oospore of Saprolegnia (normal) (Oc. 8).

27. Young antheridium of Saprolegnia, just attached to a young oangium (glycerine and sulpho-carbolate of zinc—Oc. 8).

28, 29. Two views of the oangium to which the antheridium of the preceding figure was attached; the nuclei are seen to be grouped in files (Oc. 8).

30. Oangium of Saprolegnia in early stage of segregation or "Ballung"; some nuclei elongated, one at left recalling the resting state (Oc. 12).

Figure

31. Young antheridium of Achlya (Oc. 12).

32. Male filament from which the preceding antheridium was given off (Oc. 12).

33. Rough sketch of an optical section of an intermediate stage of the oangium of Saprolegnia with a large central vacuole, and a thick peripheral layer of protoplasm containing nuclei and vacuoles (Oc. 4).

34. Oangium of Achlya at early stage of segregation; nuclei still numerous (Oc. 8, reduced $\frac{1}{2}$).

35. Part of a young oangium of Saprolegnia later than 33; the spongy cavities represent oil dissolved out in preparation (Oc. 12 ?).

36, 37, 38. Young (primary) oangial nuclei of Achlya, showing approximation and the beginning of the first fusion (Oc. 12).

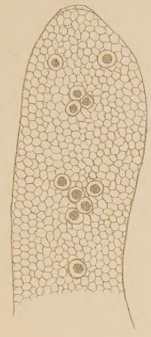
39. Secondary oangial nuclei of Achlya, formed by several fusions with multiple masses of chromatin (Oc. 12).

40. Secondary oangial nuclei, with aggregates of chromatin (Oc. 18).

41. Later secondary nuclei; *a*, *b*, two views of the same nuclei at different foci, showing two groups, each of several granules such as might be formed by the fusion of two of the nuclei shown in fig. 39; *c*, *d*, numerous chromatin granules all grouped in the centre. It will be seen how great a reduction has taken place (Oc. 18 ?).

42, 43. Surface view and optical section of oangium of Saprolegnia about same stage as fig. 33, showing relations of nuclei and vacuoles.

44. Oangium of Achlya, showing nuclei with pale walls, vacuoles, and large microsomes.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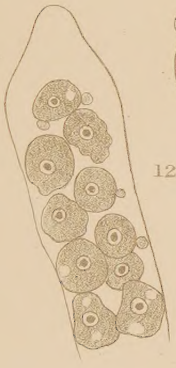
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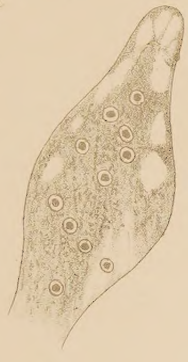
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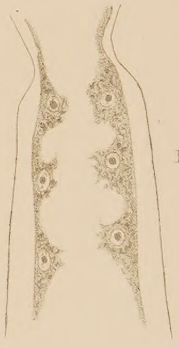
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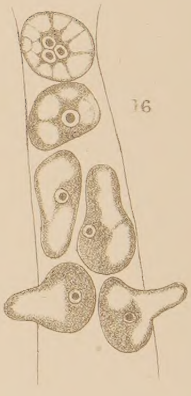
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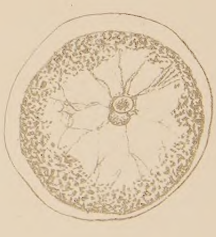
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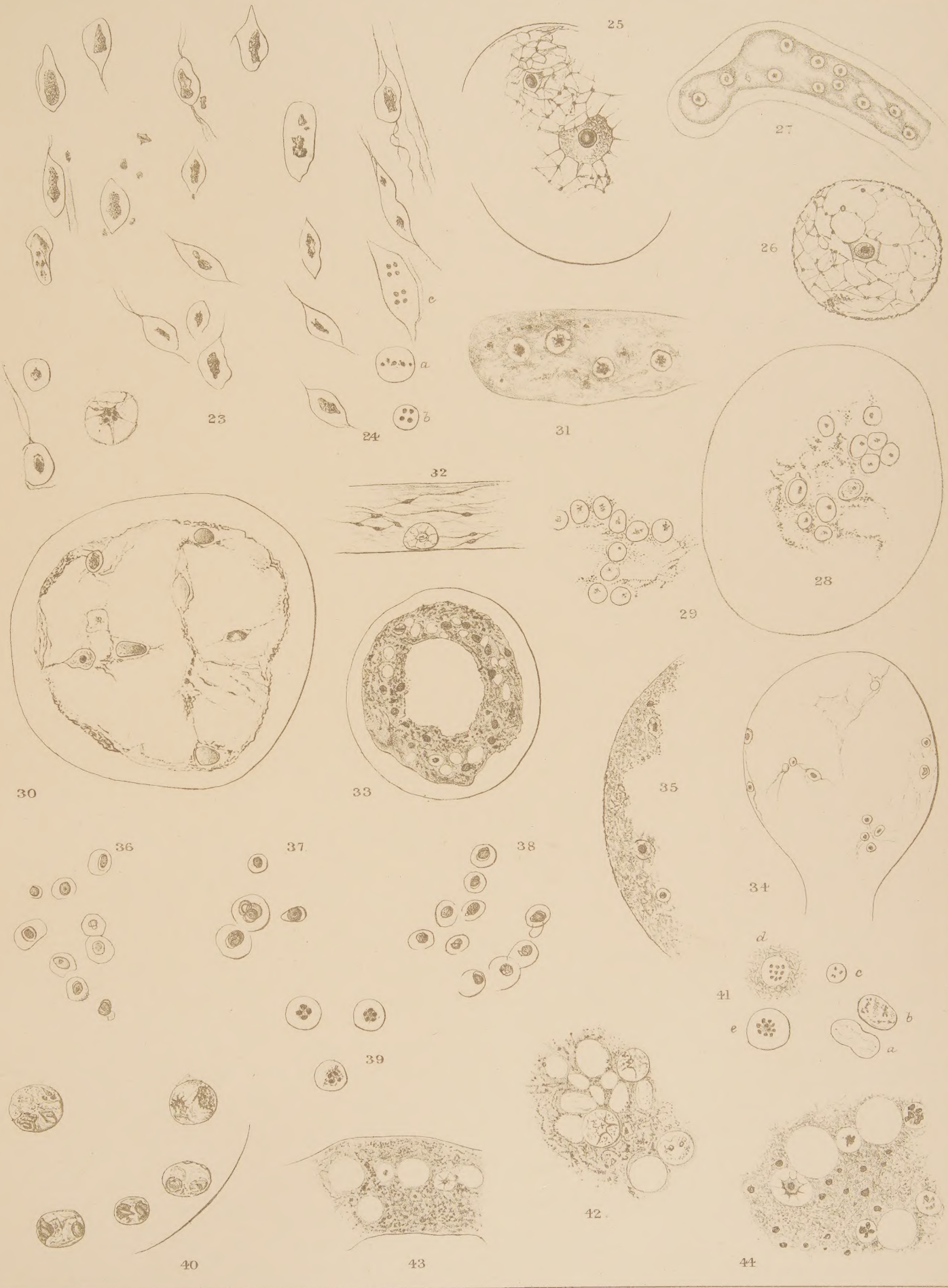
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